



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <b>A61K</b>	<b>A2</b>	(11) International Publication Number: <b>WO 97/26853</b> (43) International Publication Date: 31 July 1997 (31.07.97)
(21) International Application Number: PCT/US97/01301 (22) International Filing Date: 24 January 1997 (24.01.97)  (30) Priority Data: 08/590,494      24 January 1996 (24.01.96)      US 08/626,046      1 April 1996 (01.04.96)      US 08/626,685      1 April 1996 (01.04.96)      US 08/721,837      27 September 1996 (27.09.96)      US  (60) Parent Applications or Grants (63) Related by Continuation US                      08/721,837 (CIP) Filed on                27 September 1996 (27.09.96) US                      08/626,685 (CIP) Filed on                1 April 1996 (01.04.96) US                      08/626,046 (CIP) Filed on                1 April 1996 (01.04.96) US                      08/590,494 (CIP) Filed on                24 January 1996 (24.01.96)  (71) Applicant (for all designated States except US): SYNAPTIC PHARMACEUTICAL CORPORATION [US/US]; 215 Col- lege Road, Paramus, NJ 07652 (US).	(72) Inventors; and (75) Inventors/Applicants (for US only): SMITH, Kelli, E. [US/US]; 401 Riverside Drive, Wayne, NJ 07470 (US). GERALD, Christophe, P., G. [FR/US]; 204B Union Street, Ridgewood, NJ 07450 (US). WEINSHANK, Richard, L. [US/US]; 268 Vandelinda Avenue, Teaneck, NJ 07666 (US). LINEMEYER, David [US/US]; 105 Melba Street, Milford, CT 06460 (US). BRANCHEK, Theresa [US/US]; 518 Standish Road, Teaneck, NJ 07666 (US). FORRAY, Carlos [CL/US]; 304 Spring Valley Road, Paramus, NJ 07652 (US).  (74) Agent: WHITE, John, P.; Cooper & Dunham, L.L.P., 1185 Avenue of the Americas, New York, NY 10036 (US).  (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  Published Without international search report and to be republished upon receipt of that report.	

(54) Title: DNA ENCODING GALANIN GALR2 RECEPTORS AND USES THEREOF

## (57) Abstract

This invention provides isolated nucleic acids encoding mammalian galanin receptors, isolated galanin receptor proteins, vectors comprising isolated nucleic acid encoding a mammalian galanin receptor, cells comprising such vectors, antibodies directed to a mammalian galanin receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian galanin receptor, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding a mammalian galanin receptor, nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian galanin receptor, as well as methods of determining binding of compounds to mammalian galanin receptors.

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**DNA ENCODING GALANIN GALR2 RECEPTORS AND USES THEREOF**

5 This application is a continuation-in-part in the U.S. of  
U.S. Serial No. 08/721,837, filed September 27, 1996,  
which is a continuation-in-part of U.S. Serial No.  
08/626,685 and U.S. Serial No. 08/626,046, both filed  
10 April 1, 1996, which are continuations-in-part of U.S.  
Serial No. 08/590,494, filed January 24, 1996, the  
contents of which are hereby incorporated by reference.

**BACKGROUND OF THE INVENTION**

15 Throughout this application, various references are  
referred to within parentheses. Disclosures of these  
publications in their entireties are hereby incorporated  
by reference into this application to more fully describe  
the state of the art to which this invention pertains.  
Full bibliographic citation for these references may be  
20 found at the end of this application, preceding the  
sequence listing and the claims.

The neuropeptide galanin and its receptors hold great  
promise as targets for the development of novel  
25 therapeutic agents. Galanin is widely distributed  
throughout the peripheral and central nervous systems and  
is associated with the regulation of processes such as  
somatosensory transmission, smooth muscle contractility,  
hormone release, and feeding (for review, see Bartfai et  
30 al., 1993). In the periphery galanin is found in the  
adrenal medulla, uterus, gastrointestinal tract, dorsal  
root ganglia (DRG), and sympathetic neurons. Galanin  
released from sympathetic nerve terminals in the pancreas  
is a potent regulator of insulin release in several  
35 species (Ahrén and Lindskog, 1992; Boyle et al., 1994),  
suggesting a potential role for galanin in the etiology  
or treatment of diabetes. High levels of galanin are  
observed in human and rat anterior pituitary where  
galanin mRNA levels are potently upregulated by estrogen  
40 (Vrontakis et al., 1987; Kaplan et al., 1988). The

presence of galanin in the hypothalamic-pituitary-adrenal axis coupled with its potent hormonal effects has led to the suggestion that galanin may play an integral role in the hormonal response to stress (Bartfai et al., 1993).

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Within the CNS galanin-containing cell bodies are found in the hypothalamus, hippocampus, amygdala, basal forebrain, brainstem nuclei, and spinal cord, with highest concentrations of galanin in the hypothalamus and pituitary (Skofitsch and Jacobowitz, 1985; Bennet et al., 1991; Merchenthaler et al., 1993). The distribution of galanin receptors in the CNS generally complements that of galanin peptide, with high levels of galanin binding observed in the hypothalamus, amygdala, hippocampus, brainstem and dorsal spinal cord (Skofitsch et al., 1986; Merchenthaler et al., 1993; see Bartfai et al., 1993). Accordingly, agents modulating the activity of galanin receptors would have multiple potential therapeutic applications in the CNS. One of the most important of these is the regulation of food intake. Galanin injected into the paraventricular nucleus (PVN) of the hypothalamus stimulates feeding in satiated rats (Kyrkouli et al., 1990), an effect which is blocked by the peptide galanin antagonist M40 (Crawley et al., 1993). In freely feeding rats, PVN injection of galanin preferentially stimulates fat-preferring feeding (Tempel et al., 1988); importantly, the galanin antagonist M40 administered alone decreases overall fat intake (Leibowitz and Kim, 1992). These data indicate that specific receptors in the hypothalamus mediate the effects of galanin on feeding behavior, and further suggest that agents acting at hypothalamic galanin receptors may be therapeutically useful in the treatment of human eating disorders.

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Galanin receptors elsewhere in the CNS may also serve as therapeutic targets. In the spinal cord galanin is



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released from the terminals of sensory neurons as well as spinal interneurons and appears to play a role in the regulation of pain threshold (Wiesenfeld-Hallin et al., 1992). Intrathecal galanin potentiates the antinociceptive effects of morphine in rats and produces analgesia when administered alone (Wiesenfeld-Hallin et al., 1993; Post et al., 1988); galanin receptor agonists may therefore be useful as analgesic agents in the spinal cord. Galanin may also play a role in the development of Alzheimer's disease. In the hippocampus galanin inhibits both the release (Fisone et al., 1987) and efficacy (Palazzi et al., 1988) of acetylcholine, causing an impairment of cognitive functions (Sundström et al., 1988). Autopsy samples from humans afflicted with Alzheimer's disease reveal a galaninergic hyperinnervation of the nucleus basalis (Chan-Palay, 1988), suggesting a role for galanin in the impaired cognition characterizing Alzheimer's disease. Together these data suggest that a galanin antagonist may be effective in ameliorating the symptoms of Alzheimer's disease (see Crawley, 1993). This hypothesis is supported by the report that intraventricular administration of the peptide galanin antagonist M35 improves cognitive performance in rats (Ögren et al., 1992). Human galanin receptors thus provide targets for therapeutic intervention in multiple CNS disorders.

High-affinity galanin binding sites have been characterized in brain, spinal cord, pancreatic islets and cell lines, and gastrointestinal smooth muscle in several mammalian species, and all show similar affinity for <sup>125</sup>I-porcine galanin (-0.5-1 nM). Nevertheless, recent in vitro and in vivo pharmacological studies in which fragments and analogues of galanin were used suggest the existence of multiple galanin receptor subtypes. For example, a galanin binding site in guinea pig stomach has been reported that exhibits high affinity for porcine

galanin (3-29) (Gu, et al. 1995), which is inactive at CNS galanin receptors. The chimeric galanin analogue M15 (galantide) acts as antagonist at CNS galanin receptors (Bartfai et al., 1991) but as a full agonist in gastrointestinal smooth muscle (Gu et al., 1993). Similarly, the galanin-receptor ligand M40 acts as a weak agonist in RINm5F insulinoma cells and a full antagonist in brain (Bartfai et al., 1993a). The pharmacological profile of galanin receptors in RINm5F cells can be further distinguished from those in brain by the differential affinities of [D-Tyr<sup>2</sup>]- and [D-Phe<sup>2</sup>]-galanin analogues (Lagny-Pourmir et al., 1989). The chimeric galanin analogue M35 displaces <sup>125</sup>I-galanin binding to RINm5F membranes in a biphasic manner, suggesting the presence of multiple galanin receptor subtypes, in this cell line (Gregersen et al., 1993).

Multiple galanin receptor subtypes may also co-exist within the CNS. Galanin receptors in the dorsal hippocampus exhibit high affinity for Gal (1-15) but not for Gal (1-29) (Hedlund et al., 1992), suggesting that endogenous proteolytic processing may release bioactive fragments of galanin to act at distinct receptors. The rat pituitary exhibits high-affinity binding for <sup>125</sup>I-Bolton and Hunter (N-terminus)-labeled galanin (1-29) but not for [<sup>125</sup>I]Tyr<sup>26</sup>-porcine galanin (Wynick et al., 1993), suggesting that the pituitary galanin receptor is a C-terminus-preferring subtype. Spinal cord galanin binding sites, while similar to those in brain, show an affinity for the chimeric peptide antagonist M35 intermediate between the brain and smooth muscle (Bartfai et al., 1991), raising the possibility of further heterogeneity.

A galanin receptor cDNA was recently isolated by expression cloning from a human Bowes melanoma cell line (Habert-Ortoli et al., 1994). The pharmacological profile exhibited by this receptor is similar to that

observed in brain and pancreas, and on that basis the receptor has been termed GALR1. The cloned human GALR1 receptor binds native human, porcine and rat galanin with ~1 nM affinity ( $K_i$  vs.  $^{125}$ I-galanin) and porcine galanin 1-16 at a slightly lower affinity (~5nM). Porcine galanin 3-29 does not bind to the receptor. The GALR1 receptor appears to couple to inhibition of adenylate cyclase, with half-maximal inhibition of forskolin-stimulated cAMP production by 1 nM galanin, and maximal inhibition occurring at about 1  $\mu$ M.

Recently the rat homologue of GALR1 was cloned from the RIN14B pancreatic cell line (Burgevin, et al., 1995, Parker et al., 1995; Smith et al., in preparation). The pharmacological data reported to date do not suggest substantial differences between the pharmacologic properties of the rat and human GALR1 receptors. Localization studies reveal GALR1 mRNA in rat hypothalamus, ventral hippocampus, brainstem, and spinal cord (Gustafson et al., 1996), regions consistent with roles for galanin in feeding, cognition, and pain transmission. However, GALR1 appears to be distinct from the pituitary and hippocampal receptor subtypes described above.

The indication of multiple galanin receptor subtypes within the brain underscores the importance of defining galanin receptor heterogeneity at the molecular level in order to develop specific therapeutic agents for CNS disorders. Pharmacological tools capable of distinguishing galanin receptor subtypes in tissue preparations are only beginning to appear. Several high-affinity peptide-based galanin antagonists have been developed and are proving useful in probing the functions of galanin receptors (see Bartfai et al., 1993), but their peptide character precludes practical use as therapeutic agents. In light of galanin's multiple

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neuroendocrin roles, therapeutic agents targeting a specific disorder must be selective for the appropriate receptor subtyp to minimize side effects.

5 Accordingly, the cloning of the entire family of galanin receptors for use in target-based drug design programs has been endeavored. The identification of non-peptide agents acting selectively only at specific galanin  
10 receptors will be greatly facilitated by the cloning, expression, and characterization of the galanin receptor family.

The isolation by expression cloning of a novel galanin receptor from a rat hypothalamic cDNA library, as well as  
15 its pharmacological characterization in a heterologous expression system is now reported. The data provided demonstrate for the first time the existence of a new galanin receptor subtype, from now on referred to as the GALR2 subtype, or simply, "GALR2." The cloning of the  
20 human homolog of the rat GALR2 receptor is also reported. This discovery provides a novel approach, through the use of heterologous expression systems, to develop subtype selective, high-affinity non-peptide compounds that could serve as therapeutic agents for eating disorders,  
25 diabetes, pain, depression, ischemia, and Alzheimer's disease. The presence of both GALR1 and GALR2 in rat hypothalamus suggests that multiple galanin receptors may be involved in the regulation of feeding. Pathophysiological disorders proposed to be linked to  
30 galanin receptor activation include eating disorders, diabetes, pain, depression, ischemia, Alzheimer's disease and reproductive disorders. Accordingly, treatment of such disorders may be effected by the administration of GALR2 receptor-selective compounds. The localization of  
35 GALR2 receptors in other parts of the rat brain suggests that GALR2 receptors may play a role in cognition, analgesia, sensory processing (olfactory, visual),

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processing of visceral information, motor coordination, modulation of dopaminergic activity, neuroendocrine function, sleep disorders, migraine, and anxiety.

**SUMMARY OF THE INVENTION**

This invention provides an isolated nucleic acid encoding a mammalian GALR2 galanin receptor. This invention also provides an isolated GALR2 receptor protein. This invention further provides DNA, cDNA, genomic DNA, RNA, and mRNA encoding the GALR2 receptor.

This invention further provides a vector comprising the GALR2 receptor. This invention also provides a plasmid which comprises the regulatory elements necessary for expression of GALR2 nucleic acid in a mammalian cell operatively linked to a nucleic acid encoding the GALR2 receptor so as to permit expression thereof, designated K985 (ATCC Accession No. 97426). This invention also provides a plasmid which comprises the regulatory elements necessary for expression of GALR2 nucleic acid in a mammalian cell operatively linked to a nucleic acid encoding the GALR2 receptor so as to permit expression thereof, designated B029 (ATCC Accession No. 97735). This invention provides mammalian cells comprising the above-described plasmid or vector. This invention also provides a membrane preparation isolated from the cells.

This invention provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the GALR2 receptor contained in plasmid K985, plasmid B029, plasmid B039 or plasmid K1045. In one embodiment, the GALR2 receptor is the rat GALR2 receptor encoded by the coding sequence of plasmid K985. In another embodiment, the GALR2 receptor is the human GALR2 receptor encoded by the coding sequence of plasmid B029. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid

encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence within (a) the nucleic acid sequence shown in Figure 1 or Figure 10, or (b) the reverse complement of the nucleic acid sequence shown in Figure 1 or Figure 10. This invention further provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a GALR2 galanin receptor, so as to prevent translation of the mRNA. This invention also provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule encoding a GALR2 receptor.

This invention provides an antibody directed to a GALR2 receptor. This invention also provides a monoclonal antibody directed to an epitope of a GALR2 receptor, which epitope is present on the surface of a cell expressing a GALR2 receptor.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to reduce activity of a GALR2 receptor by passing through a cell membrane and binding specifically with mRNA encoding a GALR2 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane. In an embodiment, the oligonucleotide is coupled to a substance

which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme.

5 This invention provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

10 This invention provides a pharmaceutical composition comprising an amount of an agonist effective to increase activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

15 This invention provides a transgenic nonhuman mammal expressing DNA encoding a GALR2 receptor. This invention provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native GALR2 receptor. This invention provides a transgenic nonhuman  
20 mammal whose genome comprises antisense DNA complementary to DNA encoding a GALR2 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GALR2 receptor and which hybridizes to mRNA encoding a GALR2 receptor thereby reducing its  
25 translation.

This invention also provides a process for determining whether a compound can specifically bind to a GALR2 receptor which comprises contacting a cell transfected  
30 with and expressing DNA encoding the GALR2 receptor with the compound under conditions permitting binding of compounds to such receptor, and detecting the presence of any such compound specifically bound to the GALR2 receptor, so as to thereby determine whether the ligand  
35 specifically binds to the GALR2 receptor.

This invention provides a process for determining whether



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a compound can specifically bind to a GALR2 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the compound under conditions permitting binding of compounds to such receptor, and detecting the presence of the compound specifically bound to the GALR2 receptor, so as to thereby determine whether the compound specifically binds to the GALR2 receptor.

In one embodiment, the GALR2 receptor is a mammalian GALR2 receptor. In another embodiment, the GALR2 receptor is a rat GALR2 receptor. In still another embodiment, the GALR2 receptor has substantially the same amino acid sequence encoded by the plasmid K985. In another embodiment, the GALR2 receptor is a human GALR2 receptor. In still another embodiment, the GALR2 receptor has substantially the same amino acid sequence as the sequence encoded by plasmid B029.

This invention provides a process for determining whether a compound is a GALR2 receptor agonist which comprises contacting a cell transfected with and expressing DNA encoding the GALR2 receptor with the compound under conditions permitting the activation of the GALR2 receptor, and detecting an increase in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor agonist.

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This invention provides a process for determining whether a compound is a GALR2 receptor antagonist which comprises contacting a cell transfected with and expressing DNA encoding the GALR2 receptor with the compound in the presence of a known GALR2 receptor agonist, such as galanin, under conditions permitting the activation of the GALR2 receptor, and detecting a decrease in GALR2

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receptor activity, so as to thereby determine whether the compound is a GALR2 receptor antagonist.

5 This invention provides a compound determined by the above-described processes. In one embodiment of the above-described processes, the compound is not previously known. In another embodiment, the compound is not previously known to bind to a GALR2 receptor.

10 This invention provides a process of screening a plurality of chemical compounds not known to bind to a GALR2 receptor to identify a compound which specifically binds to the GALR2 receptor, which comprises (a) contacting cells transfected with and expressing DNA  
15 encoding the GALR2 receptor with a compound known to bind specifically to the GALR2 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the GALR2 receptor, under conditions permitting binding of compounds known to  
20 bind the GALR2 receptor; (c) determining whether the binding of the compound known to bind to the GALR2 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately  
25 determining the binding to the GALR2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the GALR2 receptor.

30 This invention provides a method of screening a plurality of chemical compounds not known to activate a GALR2 receptor to identify a compound which activates the GALR2 receptor which comprises (a) contacting cells transfected with and expressing the GALR2 receptor with the plurality  
35 of compounds not known to activate the GALR2 receptor, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activity of the

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GALR2 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the GALR2 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR2 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a GALR2 receptor to identify a compound which inhibits the activation of the GALR2 receptor, which comprises (a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds in the presence of a known GALR2 receptor agonist, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activation of the GALR2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR2 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the GALR2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the GALR2 receptor.

This invention provides a method of detecting expression of a GALR2 receptor by detecting the presence of mRNA coding for the GALR2 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the above-described nucleic acid probe under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the GALR2 receptor by the cell.

This invention provides a method of treating an

abnormality in a subject, wherein the abnormality is alleviated by the inhibition of a GALR2 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition effective to decrease the activity of the GALR2 receptor in the subject, thereby treating the abnormality in the subject. In an embodiment, the abnormality is obesity. In another embodiment, the abnormality is bulimia.

10 This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a GALR2 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition effective to activate the GALR2 receptor in the subject. In an embodiment, the abnormal condition is anorexia.

This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human GALR2 receptor allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human GALR2 receptor and labelled with a detectable marker; (e) detecting labelled bands which have hybridized to DNA encoding a human GALR2 receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; (f) preparing DNA obtained for diagnosis by steps a-e; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis

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from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

5 This invention provides a method of modifying feeding behavior of a subject which comprises administering to the subject an amount of a compound which is a galanin receptor agonist or antagonist effective to increase or decrease the consumption of food by the subject so as to  
10 thereby modify feeding behavior of the subject. In an embodiment, the compound is a GALR2 receptor antagonist and the amount is effective to decrease the consumption of food by the subject. In another embodiment the compound is administered in combination with food.

15 In yet another embodiment the compound is a GALR2 receptor agonist and the amount is effective to increase the consumption of food by the subject. In a still further embodiment, the compound is administered in  
20 combination with food. In other embodiments the subject is a vertebrate, a mammal, a human or a canine.

**BRIEF DESCRIPTION OF THE FIGURES**

**Figure 1** Nucleotide coding sequence of the rat hypothalamic galanin GALR2 receptor (Seq. I.D. No. 7), with partial 5' and 3' untranslated sequences. Start (ATG) and stop (TAA) codons are underlined.

**Figure 2** Deduced amino acid sequence of the rat hypothalamic galanin GALR2 receptor encoded by the nucleotide sequence shown in Figure 1 (Seq. I.D. No. 8).

**Figures 3A-3C** 3A. Diagram of the intron-exon arrangement of the rat GALR2 receptor cDNA contained in plasmid K985. Untranslated regions are indicated by dark hatched segments, and coding region is unmarked except for light gray hatched segments indicating the location of the transmembrane domains of the rat GALR2 receptor. The black segment indicates the location of the intron. 3B. Splice junction sequences of the rat GALR2 receptor. Nucleotide number 1 is located 45 nucleotides upstream of the start codon (Seq. I.D. No. 9). 3C. Intron sequence of rat GALR2 receptor cDNA contained in plasmid K985. Nucleotide number 1 is located 45 nucleotides upstream of the start codon (Seq. I.D. No. 9).

**Figures 4A-4C** Localization of [<sup>125</sup>I]galanin binding sites in rat CNS. **Figure 4A-1 and 4A-4:** Distribution of total [<sup>125</sup>I]galanin binding in coronal sections through the hypothalamus and amygdala. **Figures 4A-2 and 4A-5:** Binding which remains in these areas following incubation with 60 nM [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub>. **Figures 4A-3 and 4A-6:** Binding obtained after incubation with 5 μM porcine galanin, which represents the non-specific binding condition. **Figure 4B:** Figures 4B-1 to 4B-8: Higher magnification photomicrographs of the [<sup>125</sup>I]galanin binding sites in the hypothalamus and amygdala. **Figure 4B-1:** Total binding in the paraventricular hypothalamic nucleus (PVN), virtually all of which is removed by 60 nM [D-

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Trp<sup>2</sup>]galanin<sub>(1-29)</sub> (panel 3B). **Figures 4B-3 and 4B-4:** Binding in the ventromedial hypothalamus (VMH), lateral hypothalamus (LH), and zona incerta (ZI). In these regions, some [<sup>125</sup>I]galanin binding remains after incubation with 60 nM [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> (Figure 4B-4).

**Figures 4B-5 and 4B-7:** Total binding in the amygdala. After incubation with 60 nM [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> (panels 5B and 6B), the binding is markedly reduced in the piriform cortex (Pir), and to a lesser extent in the medial nucleus (Me), and central nucleus (Ce). However, the binding in the nucleus of the lateral olfactory tract (LOT) is largely unaffected. **Figure 4C: Panels 4C-1 to 4C-6:** Distribution of [<sup>125</sup>I]galanin binding sites in the anterior forebrain (panel 7) and in the midbrain (panel 8).

In the lateral septum (LS) and insular cortex (CTX), much of the total binding (panel 7A) is removed by 60 nM [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> (panel 7B). Similarly, the total binding observed in the superior colliculus (SC), central gray (CG), and pontine reticular nucleus (PnO) (panel 8A) is markedly diminished (panel 8B). **Figures 4C-3 and 4C-6:** Nonspecific binding observed in adjacent sections through the septum and midbrain. Arc, arcuate nucleus; Ce, central amygdaloid nucleus; CL, centrolateral thalamic nucleus; LOT, nucleus of the lateral olfactory tract; Me, medial amygdaloid nucleus; Pir, piriform cortex; PVN, paraventricular hypothalamic nucleus; SO, supraoptic nucleus; st, stria terminalis; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta.

**Figure 5.** Reverse transcriptase PCR (RT-PCR) of rat GALR2 receptor mRNA from various brain regions. The blot was hybridized at high stringency with an oligonucleotide probe corresponding to a portion of the predicted V/VI loop of GALR2. Positive controls are indicated by +s and represent plasmids containing the indicated inserts. Size standards are indicated at the left in kilobases. Note the additional hybridizing bands intermediate in

size between the intron-containing and the intronless product.

5 **Figures 6A-6B.** Northern blot analysis of GALR2 receptor mRNA from various rat brain regions.

6A. A Northern blot containing poly A<sup>+</sup> RNA (~5 µg) from six different rat brain regions was hybridized at high stringency with a randomly primed radiolabeled fragment representing the entire rat GALR2 coding region (not including the intron). The autoradiogram represents a four day exposure and reveals a ~1.8-2.0 kb transcript. 10 6B. The blot was reprobed with 1B15 (~1 kb) to confirm that similar amounts of RNA were present in each lane.

15 **Figures 7A-7B.** Northern blot analysis of GALR2 receptor mRNA from various rat tissues.

7A. A Northern blot containing poly A<sup>+</sup> RNA (~2 µg) from eight different rat tissues was hybridized at high stringency with a randomly primed radiolabeled fragment representing the entire rat GALR2 coding region (not including the intron). The autoradiogram represents a four day exposure and reveals a single ~ 1.8-2.0 kb transcript. 20

25 7B. The Northern blot was reprobed for 1B15 (~1 kb) to confirm that similar amounts of RNA were present in each lane. A second Northern blot (not shown) was also hybridized under the same conditions and showed similar results (Table 3).

30 **Figures 8A-8D.** Rat GALR2 receptor autoradiography in COS-7 cells transfected with GALR1 and GALR2 cDNAs. <sup>125</sup>I-[D-Trp<sup>2</sup>]Galanin<sub>(1-29)</sub> was tested as a selective radioligand for GALR2. Panels represent dark-field photomicrographs (200X) of photoemulsion-dipped slides. 8A: Binding of 3 nM <sup>125</sup>I-[D-Trp<sup>2</sup>]Galanin<sub>(1-29)</sub> to COS-7 cells transiently transfected with GALR2. Note positive binding to cells. 35



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8B: Nonspecific binding of 6 nM  $^{125}\text{I}$ -[D-Trp<sup>2</sup>]Galanin<sub>(1-29)</sub> in the presence of 300 nM porcine galanin<sub>(1-29)</sub> to COS-7 cells transiently transfected with GALR2.

8C: Binding of 6 nM  $^{125}\text{I}$ -[D-Trp<sup>2</sup>]Galanin<sub>(1-29)</sub> to COS-7 cells transiently transfected with GALR1. Note absence of binding to cell profiles; small accumulations of silver grains represent nonspecific nuclear association.

8D: Nonspecific binding of 6 nM  $^{125}\text{I}$ -[D-Trp<sup>2</sup>]Galanin<sub>(1-29)</sub> in the presence of 600 nM porcine galanin<sub>(1-29)</sub> to COS-7 cells transiently transfected with GALR1.

**Figures 9A-9B.** Functional response mediated by LM(tk-) cells stably transfected with the cDNA encoding the rat GALR2 receptor. 9A: Inhibition of cyclic AMP formation: cells were incubated with varying concentrations of porcine galanin<sub>(1-29)</sub>, and 10  $\mu\text{M}$  forskolin for 15 min. at 37°C. Data was normalized taking as 0% the basal levels of cyclic AMP ( $0.06 \pm 0.02$  pmol/ml) and 100% the cAMP levels produced by forskolin in the absence of agonist ( $0.26 \pm 0.03$  pmol/ml). Data is shown as mean  $\pm$  standard error of the mean of four independent experiments. 9B: Phosphoinositide metabolism: cells were incubated for 18 hours in the presence of 0.5  $\mu\text{Ci}$  [ $^3\text{H}$ ]myo-inositol. Eleven different concentrations of porcine galanin<sub>(1-29)</sub> were added in the presence of 10mM LiCl. Cells were incubated for 1 hour at 37°C, and [ $^3\text{H}$ ]inositol phosphates were isolated and measured.

**Figure 10.** Nucleotide coding sequence of the human galanin GALR2 receptor (Seq. I.D. No. 29), with partial 5' and 3' untranslated sequences. Start (ATG) and stop (TGA) codons are underlined.

**Figure 11.** Deduced amino acid sequence of the human galanin GALR2 receptor encoded by the nucleotide sequence shown in Figure 10 (Seq. I.D. No. 30).

**Figures 12A-12C.** 12A. Diagram of the intron-exon arrangement of the human GALR2 receptor cDNA contained in plasmid B029. Untranslated regions are indicated by dark hatched segments, and coding region is unmarked except for light gray hatched segments indicating the location of the transmembrane domains of the human GALR2 receptor. The black segment indicates the location of the intron. 12B. Splice junction sequences of the human GALR2 receptor. 12C. Intron sequence of human GALR2 receptor cDNA contained in plasmid B029 (Seq. I.D. No. 31).

**Figure 13.** Current response in an oocyte injected with 50 pg of GALR2 mRNA. Holding potential was -80 mV.

**Figure 14.** Autoradiograph demonstrating hybridization of radiolabeled rGALR2 probe to RNA extracted from rat. The lower band (arrow) represents mRNA coding for the rat GALR2 extracted from tissue indicated at the bottom of the gel. RNA coding for the rat GALR2 is present in: the heart, kidney, hypothalamus, hippocampus, amygdala, spinal cord, and cerebellum. mRNA coding for the rat GALR2 was not detected in RNA extracted from striated muscle or liver.

**Figures 15A-15D.** Amino acid sequence alignment of the rat GALR2 receptor (top row) (Seq. ID No. 8), human GALR2 receptor (middle row) (Seq. ID No. 29) and rat GALR1 receptor (bottom row) (Seq. ID No. 32). Transmembrane domains (TM 1-7) are indicated by brackets above the sequence.

**DETAILED DESCRIPTION OF THE INVENTION**

Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

5           C=cytosine                   A=adenine  
          T=thymine                   G=guanine

Furthermore, the term "agonist" is used throughout this application to indicate any peptide or non-peptidyl compound which increases the activity of any of the  
10   receptors of the subject invention. The term "antagonist" is used throughout this application to indicate any peptide or non-peptidyl compound which decreases the activity of any of the receptors of the subject invention.

15   The activity of a G-protein coupled receptor such as a galanin receptor may be measured using any of a variety of functional assays in which activation of the receptor in question results in an observable change in the level  
20   of some second messenger system, including but not limited to adenylate cyclase, calcium mobilization, arachidonic acid release, ion channel activity, inositol phospholipid hydrolysis or guanylyl cyclase. Heterologous expression systems utilizing appropriate  
25   host cells to express the nucleic acid of the subject invention are used to obtain the desired second messenger coupling. Receptor activity may also be assayed in an oocyte expression system.

30   This invention provides an isolated nucleic acid encoding a GALR2 galanin receptor. In an embodiment, the galanin receptor is a vertebrate or a mammalian GALR2 receptor. In another embodiment, the galanin receptor is a rat GALR2 receptor. In another embodiment, the galanin  
35   receptor is a human GALR2 receptor. In an embodiment, the isolated nucleic acid encodes a receptor characterized by an amino acid sequence in the

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transmembrane region, which has a homology of 60% or higher to the amino acid sequence in the transmembrane region of the rat galanin GALR2 receptor and a homology of less than 60% to the amino acid sequence in the transmembrane region of any GALR1 receptor. In one embodiment, the GALR2 receptor is a rat GALR2 receptor. In another embodiment, the GALR2 receptor is a human GALR2 receptor.

10 This invention provides an isolated nucleic acid encoding a GALR2 receptor having substantially the same amino acid sequence as shown in Figure 2. In one embodiment, the nucleic acid is DNA. This invention further provides an isolated nucleic acid encoding a rat GALR2 receptor  
15 having the amino acid sequence shown in Figure 2. In another embodiment, the nucleic acid comprises at least an intron. In yet another embodiment, the intron comprises a fragment of the intron sequence shown in Figure 3C (Seq. I.D. No. 9). In still another  
20 embodiment, the nucleic acid comprises alternately spliced nucleic acid transcribed from the nucleic acid contained in plasmid K985. In one embodiment, the alternately spliced nucleic acid is mRNA transcribed from DNA encoding a galanin receptor.

25 In one embodiment, the GALR2 receptor has substantially the same amino acid sequence as the amino acid sequence encoded by plasmid K985 (ATCC Accession No. 97426). In another embodiment, the GALR2 receptor has the amino  
30 acid sequence encoded by the plasmid K985. In still another embodiment, the GALR2 receptor has substantially the same amino acid sequence as the amino acid sequence encoded by the plasmid K1045. In yet another embodiment, the GALR2 receptor has the amino acid sequence encoded by  
35 the plasmid K1045. Plasmid K1045 comprises an intronless cDNA encoding the rat GALR2 receptor. Plasmid K1045 is further characterized by its lack of native 5' or 3'

untranslated sequences, such that the plasmid contains only the regulatory elements necessary for expression in mammalian cells (e.g., Kozak consensus sequence) and the coding sequence of the GALR2 receptor.

5 In one embodiment, the human GALR2 receptor has substantially the same amino acid sequence as the amino acid sequence encoded by plasmid BO29 (ATCC Accession No. 97735). In yet another embodiment, the human GALR2  
10 receptor has the amino acid sequence encoded by the plasmid BO29. In another embodiment, the nucleic acid encoding the human GALR2 receptor comprises an intron. In still another embodiment, the nucleic acid encoding the human GALR2 receptor comprises alternately spliced  
15 nucleic acid transcribed from the nucleic acid contained in plasmid BO29. In still another embodiment, the human GALR2 receptor has substantially the same amino acid sequence as the amino acid sequence encoded by plasmid BO39 (ATCC Accession No. ). In another embodiment,  
20 the human GALR2 receptor has the amino acid sequence encoded by the plasmid BO39. Plasmid BO39 comprises an intronless cDNA encoding the human GALR2 receptor. This invention provides an isolated nucleic acid encoding a GALR2 receptor having substantially the same amino acid  
25 sequence as shown in Figure 11 (Seq. I.D. No. 30). In one embodiment, the nucleic acid is DNA. This invention further provides an isolated nucleic acid encoding a human GALR2 receptor having the amino acid sequence shown in Figure 11.

30 The observation that both the human and rat GALR2 cDNAs contain at least one intron raises the possibility that additional introns could exist in coding or non-coding regions. In addition, spliced form(s) of mRNA may encode  
35 additional amino acids either upstream of the currently defined starting methionine or within the coding region. Further, the existence and use of alternative exons is

possible, whereby the mRNA may encode different amino acids within the region comprising the exon. In addition, single amino acid substitutions may arise via the mechanism of RNA editing such that the amino acid sequence of the expressed protein is different than that encoded by the original gene (Burns et al., 1996; Chu et al., 1996). Such variants may exhibit pharmacologic properties differing from the receptor encoded by the original gene.

10

This invention provides a splice variant of the GALR2 receptors disclosed herein. This invention further provides for alternate translation initiation sites and alternately spliced or edited variants of nucleic acids encoding rat and human GALR2 receptors.

15

This invention provides the above-described isolated nucleic acid, wherein the nucleic acid is DNA. In an embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In still another embodiment, the nucleic acid molecule is RNA. Methods for production and manipulation of nucleic acid molecules are well known in the art.

20

In another embodiment, the nucleic acid encodes a vertebrate GALR2 receptor. In a separate embodiment, the nucleic acid encodes a mammalian GALR2 receptor. In another embodiment, the nucleic acid encodes a rat GALR2 receptor. In still another embodiment, the nucleic acid encodes a human GALR2 receptor.

30

This invention further provides nucleic acid which is degenerate with respect to the DNA comprising the coding sequence of the plasmid K985. This invention also provides nucleic acid which is degenerate with respect to the DNA comprising the coding sequence of the plasmid K1045. This invention further provides nucleic acid

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which is degenerate with respect to any DNA encoding a GALR2 receptor. In one embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence described in Figure 1 (Seq. I.D. No. 1), that is, a nucleotide sequence which is translated into the same amino acid sequence. In another embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence described in Seq. I.D. No. 9.

In yet another embodiment, this invention further provides nucleic acid which is degenerate with respect to the DNA comprising the coding sequence of plasmid B029. In an embodiment, the nucleic acid comprises a nucleotide sequence which is degenerate with respect to the nucleotide sequence described in Figure 10 (Seq. I.D. No. 29), that is, a nucleotide sequence which is translated into the same amino acid sequence. This invention also provides nucleic acid which is degenerate with respect to the DNA comprising the coding sequence of the plasmid B039.

This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of the GALR2 galanin receptor, but which should not produce phenotypic changes. Alternatively, this invention also encompasses DNAs, cDNAs, and RNAs which hybridize to the DNA, cDNA, and RNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The nucleic acids of the subject invention also include nucleic acid molecules coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the

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residues specified for the protein, substitution analogs where in one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

G-protein coupled receptors such as the GALR2 receptors of the present invention are characterized by the ability of an agonist to promote the formation of a high-affinity ternary complex between the agonist, the receptor, and an intracellular G-protein. This complex is formed in the presence of physiological concentrations of GTP, and results in the dissociation of the alpha subunit of the G protein from the beta and gamma subunits of the G protein, which further results in a functional response, i.e., activation of downstream effectors such as adenylyl cyclase or phospholipase C. This high-affinity complex is transient even in the presence of GTP, so that if the complex is destabilized, the affinity of the receptor for agonists is reduced. Thus, if a receptor is not optimally coupled to G protein under the conditions of an assay, an agonist will bind to the receptor with low affinity. In contrast, the affinity of the receptor for an antagonist is normally not significantly affected by the presence or absence of G protein. Functional assays may be used to determine whether a compound binds to the receptor, but may be more time-consuming or difficult to perform than a binding assay. Therefore, it may be desirable to produce a receptor which will bind to agonists with high affinity in a binding assay. Examples



of modified receptors which bind agonists with high affinity are disclosed in WO 96/14331, which describes neuropeptide Y receptors modified in the third intracellular domain. The modifications may include deletions of 6-13 amino acids in the third intracellular loop. Such deletions preferably end immediately before the polar or charged residue at the beginning of helix six. In one embodiment, the deleted amino acids are at the carboxy terminal portion of the third intracellular domain. Such modified receptors may be produced using methods well-known in the art such as site-directed mutagenesis or recombinant techniques using restriction enzymes.

This invention provides an isolated nucleic acid encoding a modified GALR2 receptor, which differs from a GALR2 receptor by having an amino acid(s) deletion, replacement or addition in the third intracellular domain. In one embodiment, the modified GALR2 receptor differs by having a deletion in the third intracellular domain. In another embodiment, the modified GALR2 receptor differs by having an amino acid replacement or addition to the third intracellular domain.

The modified receptors of this invention may be transfected into cells either transiently or stably using methods well-known in the art, examples of which are disclosed herein. This invention also provides for binding assays using the modified receptors, in which the receptor is expressed either transiently or in stable cell lines. This invention further provides for a compound identified using a modified receptor in a binding assay such as the binding assays described herein.

The nucleic acids described and claimed herein are useful for the information which they provide concerning the

amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The nucleic acid molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

10 This invention also provides an isolated galanin GALR2 receptor protein. In one embodiment, the GALR2 receptor protein has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid K985. In another embodiment, the GALR2 receptor protein  
15 has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid K1045. In one embodiment, the GALR2 receptor protein has the same or substantially the same amino acid sequence as shown in Figure 2. In another embodiment, the GALR2  
20 receptor has the amino acid sequence shown in Figure 2. In still another embodiment, the GALR2 receptor protein has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid B029. In still another embodiment, the GALR2 receptor  
25 protein has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid B039. In an embodiment, the GALR2 receptor protein has the same or substantially the same amino acid sequence as shown in Figure 11. In another embodiment, the GALR2  
30 receptor has the amino acid sequence shown in Figure 11.

This invention provides a vector comprising the above-described nucleic acid molecule.

35 Vectors which comprise the isolated nucleic acid molecule described hereinabove also are provided. Suitable vectors comprise, but are not limited to, a plasmid or a

virus. These vectors may be transformed into a suitable host cell to form a host cell expression system for the production of a polypeptide having the biological activity of a galanin GALR2 receptor. Suitable host cells include, for example, neuronal cells such as the glial cell line C6, a Xenopus cell such as an oocyte or melanophore cell, as well as numerous mammalian cells and non-neuronal cells.

10 This invention provides the above-described vector adapted for expression in a bacterial cell which further comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding the GALR2  
15 receptor as to permit expression thereof.

This invention provides the above-described vector adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the  
20 nucleic acid in the yeast cell operatively linked to the nucleic acid encoding the GALR2 receptor as to permit expression thereof.

This invention provides the above-described vector adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the  
25 nucleic acid in the insect cell operatively linked to the nucleic acid encoding the GALR2 receptor as to permit expression thereof. In a still further embodiment, the  
30 vector is a baculovirus.

In one embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in  
35 the mammalian cell operatively linked to the nucleic acid encoding the mammalian GALR2 receptor as to permit expression thereof.

In a further embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the rat GALR2 receptor as to permit expression thereof.

10 In a still further embodiment, the vector is a plasmid.

In another embodiment, the plasmid is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the human GALR2 receptor as to permit expression thereof.

20 This invention provides the above-described plasmid adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of nucleic acid in a mammalian cell operatively linked to the nucleic acid encoding the mammalian GALR2 receptor as to permit expression thereof.

25 This invention provides a plasmid designated K985 (ATCC Accession No. 97426) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR2 galanin receptor so as to permit expression thereof.

30 This plasmid (K985) was deposited on January 24, 1996, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97426.

This invention provides a plasmid designated BO29 (ATCC Accession No. 97735) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR2 galanin receptor as to permit expression thereof.

This plasmid (BO29) was deposited on September 25, 1996, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97735.

This invention provides a plasmid designated K1045 (ATCC Accession No. 97778) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR2 galanin receptor so as to permit expression thereof.

This plasmid (K1045) was deposited on October 30, 1996, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 97426.

This invention provides a plasmid designated BO39 (ATCC Accession No. \_\_\_\_\_) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the GALR2 galanin receptor as to permit expression thereof.

This plasmid (BO39) was deposited on January 15, 1997, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under

the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. \_\_\_\_\_.

5

This invention further provides for any vector or plasmid which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a  
10 vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the receptor depending upon the host cell used. In an embodiment, the vector or plasmid comprises the coding sequence of the GALR2 receptor and the regulatory elements necessary for  
15 expression in the host cell.

This invention provides a eukaryotic cell comprising the above-described plasmid or vector. This invention provides a mammalian cell comprising the above-described  
20 plasmid or vector. In an embodiment the cell is a *Xenopus* oocyte or melanophore cell. In an embodiment, the cell is a neuronal cell such as the glial cell line C6. In an embodiment, the mammalian cell is non-neuronal in origin. In an embodiment, the mammalian cell is a  
25 COS-7 cell. In another embodiment the mammalian cell is a Chinese hamster ovary (CHO) cell. In another embodiment, the cell is a mouse Y1 cell.

In still another embodiment, the mammalian cell is a 293  
30 human embryonic kidney cell. In still another embodiment, the mammalian cell is a NIH-3T3 cell. In another embodiment, the mammalian cell is an LM(tk-) cell. In still another embodiment, the mammalian cell is the LM(tk-) cell designated L-rGALR2-8. This cell line  
35 was deposited with the ATCC on March 28, 1996, under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the

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Purposes of Patent Procedure, and was accorded ATCC Accession No. CRL-12074. In yet another embodiment, the mammalian cell is the LM(tk-) cell designated L-rGALR2I-4 (which comprises the intronless plasmid K1045). This cell line was deposited with the ATCC on October 30, 1996, under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and was accorded ATCC Accession No. CRL-12223.

In another embodiment, the mammalian cell is the Chinese hamster ovary (CHO) cell designated C-rGalR2-79. C-rGalR2-79 expresses the rat GALR2 receptor and comprises a plasmid containing the intron within the coding region. This cell line was deposited with the ATCC on January 15, 1997, under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and was accorded ATCC Accession No. CRL-12262.

This invention also provides an insect cell comprising the above-described vector. In an embodiment, the insect cell is an Sf9 cell. In another embodiment, the insect cell is an Sf21 cell.

This invention provides a membrane preparation isolated from any of the above-described cells.

This invention provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the GALR2 receptor contained in plasmid K985.

This invention further provides a nucleic acid probe

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comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the GALR2 receptor contained in plasmid K1045.

This invention still further provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence described in Figure 1 or (b) the reverse complement thereto. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the GALR2 receptor contained in plasmid B029. This invention also provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the GALR2 receptor contained in plasmid B039.

This invention provides a nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 10 (Seq. ID No. 29) or (b) the reverse complement to the nucleic acid sequence shown in Figure 10.

This invention provides a nucleic acid probe comprising



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at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within (a) the nucleic acid sequence shown in Figure 1 (Seq. I.D. No. 7) or (b) the reverse complement to the nucleic acid sequence shown in Figure 1 (Seq. I.D. No. 7). In one embodiment, the nucleic acid encoding a GALR2 receptor comprises an intron, the sequence of which intron is described in Figure 3 (Seq. I.D. No. 9). In another embodiment, the nucleic acid encoding a GALR2 receptor comprises an intron, the sequence of which intron is described in Figure 12C (Seq. I.D. No. 31).

This invention further provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor.

In one embodiment, the nucleic acid probe is DNA. In another embodiment the nucleic acid probe is RNA. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

This nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid encoding the GALR2 galanin receptors can be used as a probe. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that

such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule which encodes the GALR2 receptor into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

RNA probes may be generated by inserting the DNA molecule which encodes the GALR2 galanin receptor downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a GALR2 galanin receptor, so as to prevent translation of the mRNA.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule encoding a GALR2 receptor.

This invention provides an antisense oligonucleotide comprising chemical analogues of nucleotides.

This invention provides an antibody directed to a GALR2 receptor. This invention also provides an antibody directed to a rat GALR2 receptor. This invention also provides an antibody directed to a human GALR2 receptor. In an embodiment, the human GALR2 has an amino acid

sequence the same or substantially the same as an amino acid sequence encoded by plasmid K985 or an amino acid sequence encoded by plasmid B029. In another embodiment, the human GALR2 has an amino acid sequence the same or substantially the same as an amino acid sequence encoded by plasmid B039.

This invention provides a monoclonal antibody directed to an epitope of a GALR2 receptor, which epitope is present on the surface of a cell expressing a GALR2 receptor.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to reduce activity of a GALR2 receptor by passing through a cell membrane and binding specifically with mRNA encoding a GALR2 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane. In one embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme.

This invention provides the above-described pharmaceutical composition, wherein the pharmaceutically acceptable carrier capable of passing through a cell membrane comprises a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type.

This invention provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

This invention provides a pharmaceutical composition comprising an amount of an agonist effective to increase activity of a GALR2 receptor and a pharmaceutically

acceptable carrier.

5 This invention provides the above-described pharmaceutical composition which comprises an amount of the antibody effective to block binding of a ligand to the GALR2 receptor and a pharmaceutically acceptable carrier.

10 As used herein, "pharmaceutically acceptable carriers" means any of the standard pharmaceutically acceptable carriers. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water and emulsions, such as oil/water emulsions.

15 This invention provides a transgenic nonhuman mammal expressing DNA encoding a GALR2 receptor.

20 This invention provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native GALR2 receptor.

25 This invention provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a GALR2 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GALR2 receptor and which hybridizes to mRNA encoding a GALR2 receptor thereby reducing its translation.

30 This invention provides the above-described transgenic nonhuman mammal, wherein the DNA encoding a GALR2 receptor additionally comprises an inducible promoter.

35 This invention provides the transgenic nonhuman mammal, wherein the DNA encoding a GALR2 receptor additionally comprises tissue specific regulatory elements.

In an embodiment, the transgenic nonhuman mammal is a mouse.

Animal model systems which elucidate the physiological and behavioral roles of GALR2 receptor are produced by creating transgenic animals in which the activity of the GALR2 receptor is either increased or decreased, or the amino acid sequence of the expressed GALR2 receptor is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a GALR2 receptor, by microinjection, electroporation, retroviral transfection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal or 2) Homologous recombination of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these GALR2 receptor sequences. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native GALR2 receptors but does express, for example, an inserted mutant GALR2 receptor, which has replaced the native GALR2 receptor in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added GALR2 receptors, resulting in overexpression of the GALR2 receptors.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium. DNA or cDNA

encoding a GALR2 receptor is purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively, or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

This invention provides a process for identifying a chemical compound which specifically binds to a GALR2 receptor which comprises contacting cells containing DNA encoding and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the GALR2 receptor.

This invention further provides a process for identifying a chemical compound which specifically binds to a GALR2 receptor which comprises contacting a membrane fraction from a cell extract of cells containing DNA encoding and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with the compound under conditions suitable for

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binding, and detecting specific binding of the chemical compound to the GALR2 receptor.

5 This invention also provides a method for determining whether a chemical compound can specifically bind to a GALR2 receptor which comprises contacting cells transfected with and expressing DNA encoding the GALR2 receptor with the compound under conditions permitting binding of compounds to such receptor, and detecting the  
10 presence of any such compound specifically bound to the GALR2 receptor, so as to thereby determine whether the ligand specifically binds to the GALR2 receptor.

15 This invention provides a method for determining whether a chemical compound can specifically bind to a GALR2 receptor which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the  
20 compound under conditions permitting binding of compounds to such receptor, and detecting the presence of the compound specifically bound to the GALR2 receptor, so as to thereby determine whether the compound specifically binds to the GALR2 receptor.

25 In one embodiment, the GALR2 receptor is a mammalian GALR2 receptor. In another embodiment, the GALR2 receptor is a rat GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or  
30 substantially the same amino acid sequence as that encoded by plasmid K985, or plasmid K1045. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet  
35 another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8).

In another embodiment, the GALR2 receptor is a human GALR2 receptor. In still another embodiment, the human GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid B029 or plasmid B039. In yet another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30). In another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30).

In one embodiment, the above process further comprises determining whether the compound selectively binds to the GALR2 receptor relative to another galanin receptor. In another embodiment, the determination whether the compound selectively binds to the GALR2 receptor comprises: (a) determining the binding affinity of the compound for the GALR2 receptor and for such other galanin receptor; and (b) comparing the binding affinities so determined, the presence of a higher binding affinity for the GALR2 receptor than for such other galanin receptor indicating that the compound selectively binds to the GALR2 receptor. In an embodiment, the other galanin receptor is a GALR1 receptor. In another embodiment, the other galanin receptor is a GALR3 receptor.

This invention provides a process for determining whether a chemical compound is a GALR2 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the GALR2 receptor with the compound under conditions permitting the activation of the GALR2 receptor, and detecting an increase in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor agonist.

This invention provides a process for determining whether



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a chemical compound is a GALR2 receptor agonist which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membran fraction from the cell extract, contacting the membrane fraction with the compound under conditions permitting the activation of the GALR2 receptor, and detecting an increase in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor agonist.

In one embodiment, the GALR2 receptor is a mammalian GALR2 receptor. In another embodiment, the GALR2 receptor is a rat GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as that encoded by plasmid K985, or plasmid K1045. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8).

In another embodiment, the GALR2 receptor is a human GALR2 receptor. In still another embodiment, the human GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid BO29 or plasmid BO39. In yet another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30). In another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30).

This invention provides a process for determining whether a chemical compound is a GALR2 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the GALR2 receptor with the

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compound in the presence of a known GALR2 receptor agonist, such as galanin, under conditions permitting the activation of the GALR2 receptor, and detecting a decrease in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor antagonist.

This invention provides a process for determining whether a chemical compound is a GALR2 receptor antagonist which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known GALR2 receptor agonist, such as galanin, under conditions permitting the activation of the GALR2 receptor, and detecting a decrease in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor antagonist.

In one embodiment, the GALR2 receptor is a mammalian GALR2 receptor. In another embodiment, the GALR2 receptor is a rat GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as that encoded by plasmid K985, or plasmid K1045. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8).

In another embodiment, the GALR2 receptor is a human GALR2 receptor. In still another embodiment, the human GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid B029 or plasmid B039. In yet another embodiment, the GALR2 receptor has the same or substantially the same

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amino acid sequence as the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30). In another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30).

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In an embodiment of the above-described methods, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In a further embodiment, the cell is non-neuronal in origin. In still further embodiments, the non-neuronal cell is a COS-7 cell, 293 human embryonic kidney cell, NIH-3T3 cell, a CHO cell, or LM(tk-) cell. In yet another embodiment of any of the processes of this invention the cell is the LM(tk-) cell L-rGALR2-8 (ATCC Accession No. CRL-12074), the LM(tk-) cell L-rGALR2I-4 (ATCC Accession No. CRL-12223, or the CHO cell C-rGalR2-79 (ATCC Accession No. CRL-12262).

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This invention provides a compound determined by the above-described processes. In one embodiment of the above-described processes, the compound is not previously known to bind to a GALR2 receptor.

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This invention provides a GALR2 agonist determined by the above-described processes. This invention also provides a GALR2 antagonist determined by the above-described processes.

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This invention provides a pharmaceutical composition which comprises an amount of a GALR2 receptor agonist effective to increase activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

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This invention provides a pharmaceutical composition which comprises an amount of a GALR2 receptor antagonist effective to reduce activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

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In further embodiments of the above-described process, the agonist or antagonist is not previously known to bind to a GALR2 receptor.

5 This invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a GALR2 receptor, which comprises separately contacting cells expressing on their cell surface the GALR2 receptor, wherein such cells do not  
10 normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical  
15 compound to the GALR2 receptor, a decrease in the binding of the second chemical compound to the GALR2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR2 receptor.

20 This invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a human GALR2 receptor, which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface  
25 the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds,  
30 and detecting specific binding of the chemical compound to the GALR2 receptor, a decrease in the binding of the second chemical compound to the GALR2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR2 receptor.

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This invention further provides a process for determining whether a chemical compound specifically binds to and

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activates a GALR2 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with the chemical compound under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GALR2 receptor.

This invention further provides a process for determining whether a chemical compound specifically binds to and activates a GALR2 receptor, which comprises contacting a membrane fraction from a cell extract of cells producing a second messenger response and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with the chemical compound under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GALR2 receptor.

In one embodiment of the above processes, the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a decrease in adenylate cyclase activity. In one embodiment, adenylate cyclase activity is determined by measurement of cyclic AMP levels.

In another embodiment of the above processes, the second messenger response comprises arachidonic acid release and the change in second messenger response is an increase in

arachidonic acid levels.

5 In another embodiment of the above processes, the second messenger response comprises intracellular calcium levels and the change in second messenger response is an increase in intracellular calcium levels.

10 In a still further embodiment of the above processes, the second messenger response comprises inositol phospholipid hydrolysis and the change in second messenger response is an increase in inositol phospholipid hydrolysis.

15 This invention further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a GALR2 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a  
20 second chemical compound known to activate the GALR2 receptor, and with only the second compound, under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the  
25 presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating  
30 that the chemical compound inhibits activation of the GALR2 receptor.

35 This invention further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a GALR2 receptor, which comprises separately contacting a membrane fraction from a cell extract of cells producing a second messenger response

and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to activate the GALR2 receptor, and with only the second chemical compound, under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GALR2 receptor.

In one embodiment of the above processes, the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a smaller decrease in the level of adenylate cyclase activity in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In one embodiment, adenylate cyclase activity is determined by measurement of cyclic AMP levels.

In another embodiment of the above processes the second messenger response comprises arachidonic acid release, and the change in second messenger response is a smaller increase in arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In another embodiment of the above processes the second messenger response comprises intracellular calcium levels, and the change in second messenger response is a

smaller increase in intracellular calcium levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

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In yet another embodiment of the above processes, the second messenger response comprises inositol phospholipid hydrolysis, and the change in second messenger response is a smaller increase in inositol phospholipid hydrolysis in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

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In an embodiment of any of the above processes, the GALR2 receptor is a mammalian GALR2 receptor. In another embodiment of the above processes, the GALR2 receptor is a rat GALR2 receptor or a human GALR2 receptor. In still another embodiment of the above processes, the GALR2 receptor has the same or substantially the same amino acid sequence as encoded by the plasmid K985 (ATCC Accession No. 97426), or plasmid K1045 (ATCC Accession No. 97778). In a still further embodiment of the above processes, the GALR2 receptor has the same or substantially the same amino acid sequence as that shown in Figure 2 (Seq. ID No. 8). In another embodiment of the above processes, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by the plasmid B029 (ATCC Accession No. 97735) or the plasmid B039 (ATCC Accession No. \_\_\_\_). In a still further embodiment of the above processes, the GALR2 receptor has the same or substantially the same amino acid sequence as that shown in Figure 11 (Seq. ID No. 30).

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In an embodiment of any of the above processes, the cell is an insect cell. In another embodiment of any of the above processes, the cell is a mammalian cell. In still



further embodiments, the cell is nonneuronal in origin.

In another embodiment of the above processes, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, NIH-3T3 cell, a mouse Y1 cell or LM(tk-) cell. In still further embodiments, nonneuronal cell is the LM(tk-) cell designated L-rGALR2-8 (ATCC Accession No. CRL-12074), the LM(tk-) cell L-rGALR2I-4 (ATCC Accession No. CRL-12223, or the CHO cell C-rGalR2-79 (ATCC Accession No. \_\_\_\_\_).

This invention further provides a compound determined by any of the above processes. In another embodiment, the compound is not previously known to bind to a GALR2 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a GALR2 receptor to identify a compound which specifically binds to the GALR2 receptor, which comprises (a) contacting cells transfected with and expressing DNA encoding the GALR2 receptor with a compound known to bind specifically to the GALR2 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the GALR2 receptor, under conditions permitting binding of compounds known to bind the GALR2 receptor; (c) determining whether the binding of the compound known to bind to the GALR2 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the GALR2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the GALR2 receptor.

This invention provides a method of screening a plurality

of chemical compounds not known to bind to a GALR2 receptor to identify a compound which specifically binds to the GALR2 receptor, which comprises (a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a compound known to bind specifically to the GALR2 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the GALR2 receptor, under conditions permitting binding of compounds known to bind the GALR2 receptor; (c) determining whether the binding of the compound known to bind to the GALR2 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the GALR2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the GALR2 receptor.

In one embodiment of the above-described methods, the GALR2 receptor is a rat GALR2 receptor. In another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8). In another embodiment, the GALR2 receptor is a human GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid B029 or plasmid B039. In another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in

Figure 11 (Seq. I.D. No. 30).

This invention provides a method of screening a plurality of chemical compounds not known to activate a GALR2 receptor to identify a compound which activates the GALR2 receptor which comprises (a) contacting cells transfected with and expressing the GALR2 receptor with the plurality of compounds not known to activate the GALR2 receptor, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activity of the GALR2 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the GALR2 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR2 receptor.

This invention provides a method of screening a plurality of chemical compounds not known to activate a GALR2 receptor to identify a compound which activates the GALR2 receptor which comprises (a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds not known to activate the GALR2 receptor, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activity of the GALR2 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the GALR2 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR2 receptor.

In an embodiment of the above-described methods, the GALR2 receptor is a rat GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or

substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8). In another embodiment, the GALR2 receptor is a human GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid B029 or plasmid B039. In another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30).

15 This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a GALR2 receptor to identify a compound which inhibits the activation of the GALR2 receptor, which comprises (a) contacting cells transfected with and expressing the GALR2 receptor with the plurality of compounds in the presence of a known GALR2 receptor agonist, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activation of the GALR2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR2 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the GALR2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the GALR2 receptor.

35 This invention provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a GALR2 receptor to identify a compound which inhibits the activation of the GALR2 receptor, which comprises (a)

preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds in the presence of a known GALR2 receptor agonist, under conditions permitting activation of the GALR2 receptor; (b) determining whether the activation of the GALR2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR2 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the GALR2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the GALR2 receptor.

In an embodiment of the above-described methods, the GALR2 receptor is a rat GALR2 receptor. In another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 2 (Seq. I.D. No.8). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 2 (Seq. I.D. No. 8). In another embodiment, the GALR2 receptor is a human GALR2 receptor. In still another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence encoded by plasmid BO29 or plasmid BO39. In another embodiment, the GALR2 receptor has the same or substantially the same amino acid sequence as the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30). In yet another embodiment, the GALR2 receptor has the amino acid sequence shown in Figure 11 (Seq. I.D. No. 30).

In one embodiment of any of the above-described methods, the activation of the GALR2 receptor is determined by a second messenger assay. In an embodiment, the second

messenger assay measures adenylate cyclase activity. In other embodiments, the second messenger is cyclic AMP, intracellular calcium, or arachidonic acid or a phosphoinositol lipid metabolite. Second messenger coupling may also be measured by assaying the binding of GTP gamma S to membranes.

This invention further provides a method of measuring GALR2 receptor activation in an oocyte expression system such as a *Xenopus* oocyte or melanophore. In an embodiment, receptor activation is determined by measurement of ion channel activity.

Expression of genes in *Xenopus* oocytes is well known in the art (A. Coleman, Transcription and Translation: A Practical Approach (B.D. Hanes, S.J. Higgins, eds., pp 271-302, IRL Press, Oxford, 1984; Y. Masu et al., Nature 329:21583-21586, 1994) and is performed using microinjection of native mRNA or in vitro synthesized mRNA into frog oocytes. The preparation of in vitro synthesized mRNA can be performed by various standard techniques (J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989) including using T7 polymerase with the mCAP RNA capping kit (Stratagene).

In a further embodiment of the invention, the cell is a mammalian cell. In another embodiment of the invention, the mammalian cell is non-neuronal in origin. In still further embodiments of the invention, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a mouse Y1 cell, a LM(tk-) cell, a CHO cell, or an NIH-3T3 cell. In an embodiment of the invention, the nonneuronal cell is the LM(tk-) cell designated L-rGALR2-8 (ATCC Accession No. CRL-12074), the LM(tk-) cell L-rGALR2I-4 (ATCC Accession No. CRL-12223, or the CHO cell

C-rGalR2-79 (ATCC Accession No. \_\_\_\_\_).

5 This invention provides a pharmaceutical composition comprising a compound identified by the above-described methods and a pharmaceutically acceptable carrier.

10 In an embodiment of the above-described methods, the cell is non-neuronal in origin. In a further embodiment, the non-neuronal cell is a COS-7 cell, 293 human embryonic kidney cell, NIH-3T3 cell, a mouse Y1 cell or LM(tk-) cell.

15 In one embodiment of the above-described methods, the compound is not previously known.

20 This invention provides a GALR2 receptor agonist detected by the above-described methods. This invention provides a GALR2 receptor antagonist detected by the above-described methods. In an embodiment the cell is a non-mammalian cell, for example, a Xenopus oocyte or melanophore. In another embodiment the cell is a neuronal cell, for example, a glial cell line such as C6. In an embodiment, the cell is non-neuronal in origin. In a further embodiment, the cell is a Cos-7 or a CHO cell, 25 a 293 human embryonic kidney cell, an LM(tk-) cell or an NIH-3T3 cell. In an embodiment of the invention, the LM(tk-) cell is the cell designated L-rGALR2-8 (ATCC Accession No. CRL-12074), the LM(tk-) cell L-rGALR2I-4 (ATCC Accession No. CRL-12223, or the CHO cell C-rGalR2-30 79 (ATCC Accession No. \_\_\_\_\_).

35 This invention provides a pharmaceutical composition comprising a drug candidate identified by the above-described methods and a pharmaceutically acceptable carrier.

This invention provides a method for determining whether

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a chemical compound is a GALR2 antagonist which comprises: (a) administering to an animal a GALR2 agonist and measuring the amount of food intake in the animal; (b) administering to a second animal both the GALR2 agonist and the chemical compound, and measuring the amount of food intake in the second animal; and (c) determining whether the amount of food intake is reduced in the presence of the chemical compound relative to the amount of food intake in the absence of the compound, so as to thereby determine whether the compound is a GALR2 antagonist. This invention further provides a method of screening a plurality of chemical compounds to identify a chemical compound which is a GALR2 antagonist which comprises: (a) administering to an animal a GALR2 agonist and measuring the amount of food intake in the animal; (b) administering to a second animal the GALR2 agonist and at least one chemical compound of the plurality of compounds, and measuring the amount of food intake in the animal; (c) determining whether the amount of food intake is reduced in the presence of at least one chemical compound of the plurality of chemical compounds relative to the amount of food intake in the absence of at least one of the compounds, and if so; (d) separately determining whether each chemical compound is a GALR2 antagonist according to the method described above, so as to thereby determine if the chemical compound is a GALR2 antagonist. In one embodiment the GALR2 agonist is [D-Trp]<sub>2</sub>-galanin<sub>(1-29)</sub>. In another embodiment the animal is a non-human mammal. In a further embodiment, the animal is a rodent.

This invention provides a method of detecting expression of a GALR2 receptor by detecting the presence of mRNA coding for the GALR2 receptor which comprises obtaining total mRNA from a cell or tissue sample and contacting the mRNA so obtained with the above-described nucleic acid probe under hybridizing conditions, detecting the



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presence of mRNA hybridized to the probe, and thereby detecting the expression of the GALR2 receptor by the cell or in th tissue.

5 This invention provides a method of treating an abnormality in a subject, wherein the abnormality is alleviated by administering to the subject an amount of a GALR2 selective compound, effective to treat the abnormality. Abnormalities which may be treated include  
10 cognitive disorder, pain, sensory disorder (olfactory, visual), motor coordination abnormality, motion sickness, neuroendocrine disorders, sleep disorders, migraine, Parkinson's disease, hypertension, heart failure, convulsion/epilepsy, traumatic brain injury, diabetes,  
15 glaucoma, electrolyte imbalances, respiratory disorders (asthma, emphysema), depression, reproductive disorders, gastric and intestinal ulcers, gastroesophageal reflux disorder, gastric hypersecretion, gastrointestinal motility disorders (diarrhea), inflammation, immune  
20 disorders, and anxiety. In one embodiment the compound is an agonist. In another embodiment the compound is an antagonist.

This invention provides a method of treating an  
25 abnormality in a subject, wherein the abnormality is alleviated by the inhibition of a GALR2 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition effective to decrease the activity of the GALR2 receptor  
30 in the subject, thereby treating the abnormality in the subject. In an embodiment, the abnormality is obesity. In another embodiment, the abnormality is bulimia.

This invention provides a method of treating an  
35 abnormality in a subject wherein the abnormality is alleviated by the activation of a GALR2 receptor which comprises administering to a subject an effective amount

of the above-described pharmaceutical composition effective to activate the GALR2 receptor in the subject. In an embodiment, the abnormal condition is anorexia.

5 In another embodiment, the compound binds selectively to a GALR2 receptor. In yet another embodiment, the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which  
10 the compound binds to a GALR1 receptor. In a still further embodiment, the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR3 receptor.

15 This invention provides a method of detecting the presence of a GALR2 receptor on the surface of a cell which comprises contacting the cell with the above-described antibody under conditions permitting binding of  
20 the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a GALR2 receptor on the surface of the cell.

This invention provides a method of determining the physiological effects of varying levels of activity of  
25 GALR2 receptors which comprises producing a transgenic nonhuman mammal whose levels of GALR2 receptor activity are varied by use of an inducible promoter which regulates GALR2 receptor expression.

30 This invention provides a method of determining the physiological effects of varying levels of activity of GALR2 receptors which comprises producing a panel of transgenic nonhuman mammals each expressing a different amount of GALR2 receptor.

35 This invention provides a method for identifying an antagonist capable of alleviating an abnormality wherein

the abnormality is alleviated by decreasing the activity of a GALR2 receptor comprising administering a compound to the above-described transgenic nonhuman mammal and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overactivity of a GALR2 receptor, the alleviation of the abnormality identifying the compound as an antagonist.

This invention provides an antagonist identified by the above-described methods. This invention provides a pharmaceutical composition comprising an antagonist identified by the above-described methods and a pharmaceutically acceptable carrier.

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a GALR2 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition, thereby treating the abnormality.

This invention provides a method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR2 receptor comprising administering a compound to a transgenic nonhuman mammal and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal, the alleviation of the abnormality identifying the compound as an agonist.

This invention provides an agonist identified by the above-described methods.

This invention provides a pharmaceutical composition comprising an agonist identified by the above-described

methods and a pharmaceutically acceptable carrier.

5 This invention provides a method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR2 receptor which comprises administering to a subject an effective amount of the above-described pharmaceutical composition, thereby treating the abnormality.

10 This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific human GALR2 receptor allele which comprises: (a) obtaining DNA of subjects suffering from the disorder; (b) performing a restriction digest of the  
15 DNA with a panel of restriction enzymes; (c) electrophoretically separating the resulting DNA fragments on a sizing gel; (d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the  
20 sequence of a nucleic acid molecule encoding a human GALR2 receptor and labelled with a detectable marker; (e) detecting labelled bands which have hybridized to DNA encoding a human GALR2 receptor labelled with a detectable marker to create a unique band pattern  
25 specific to the DNA of subjects suffering from the disorder; (f) preparing DNA obtained for diagnosis by steps a-e; and (g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis  
30 from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

35 In an embodiment, a disorder associated with the activity of a specific human GALR2 receptor allele is diagnosed. In another embodiment, the above-described method may be used to identify a population of patients having a

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specific GALR2 receptor allele , in which population the disorder may be alleviated by administering to the subjects a GALR2-selective compound.

5 This invention provides a method of preparing the purified GALR2 receptor which comprises: (a) inducing cells to express GALR2 receptor; (b) recovering the receptor from the induced cells; and (c) purifying the receptor so recovered.

10

This invention provides a method of preparing a purified GALR2 receptor which comprises: (a) inserting nucleic acid encoding the GALR2 receptor in a suitable vector; (b) introducing the resulting vector in a suitable host cell; (c) placing the resulting cell in suitable condition permitting the production of the isolated GALR2 receptor; (d) recovering the receptor produced by the resulting cell; and (e) purifying the receptor so recovered.

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This invention provides a method of modifying feeding behavior of a subject which comprises administering to the subject an amount of a compound which is a galanin receptor agonist or antagonist effective to increase or decrease the consumption of food by the subject so as to thereby modify feeding behavior of the subject. In one embodiment, the compound is a GALR2 receptor antagonist and the amount is effective to decrease the consumption of food by the subject. In another embodiment the compound is administered in combination with food.

25

30

In yet another embodiment the compound is a GALR2 receptor agonist and the amount is effective to increase the consumption of food by the subject. In a still further embodiment, the compound is administered in combination with food. In other embodiments the subject is a vertebrate, a mammal, a human or a canine.

35

In one embodiment, th compound binds selectively to a GALR2 receptor. In another embodiment, the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR1 receptor. In another embodiment, the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR3 receptor. In yet another embodiment, the compound binds to the GALR2 receptor with an affinity greater than one hundred-fold higher than the affinity with which the compound binds to a GALR1 receptor. In another embodiment, the compound binds to the GALR2 receptor with an affinity greater than one hundred-fold higher than the affinity with which the compound binds to a GALR3 receptor.

This invention provides a method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a galanin receptor antagonist effective to treat the subject's Alzheimer's disease. In one embodiment, the galanin receptor antagonist is a GALR2 receptor antagonist and the amount of the compound is effective to treat the subject's Alzheimer's disease.

This invention provides a method of producing analgesia in a subject which comprises administering to the subject an amount of a compound which is a galanin receptor agonist effective to produce analgesia in the subject. In another embodiment, the galanin receptor agonist is a GALR2 receptor agonist and the amount of the compound is effective to produce analgesia in the subject.

This invention provides a method of decreasing nociception in a subject which comprises administering to th subject an amount of a compound which is a GALR2 receptor agonist effective to decrease nociception in the

subject.

5 This invention provides a method of treating pain in a subject which comprises administering to the subject an amount of a compound which is a GALR2 receptor agonist effective to treat pain in the subject.

10 This invention provides a method of decreasing feeding behavior of a subject which comprises administering a compound which is a GALR2 receptor antagonist and a compound which is a Y5 receptor antagonist, the amount of such antagonists being effective to decrease the feeding behavior of the subject. In one embodiment, the GALR2 antagonist and the Y5 antagonist are administered in  
15 combination. In another embodiment, the GALR2 antagonist and the Y5 antagonist are administered once. In another embodiment, the GALR2 antagonist and the Y5 antagonist are administered separately. In still another embodiment, the GALR2 antagonist and the Y5 antagonist  
20 are administered once. In another embodiment, the galanin receptor antagonist is administered for about 1 week to 2 weeks. In another embodiment, the Y5 receptor antagonist is administered for about 1 week to 2 weeks.

25 In yet another embodiment, the GALR2 antagonist and the Y5 antagonist are administered alternately. In another embodiment, the GALR2 antagonist and the Y5 antagonist are administered repeatedly. In a still further embodiment, the galanin receptor antagonist is  
30 administered for about 1 week to 2 weeks. In another embodiment, the Y5 receptor antagonist is administered for about 1 week to 2 weeks.

35 This invention also provides a method as described above, wherein the compound is administered in a pharmaceutical composition comprising a sustained release formulation.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

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## Experimental Details

### Materials and Methods

#### 5     Construction and screening of a rat hypothalamus cDNA library

Total RNA was prepared from rat hypothalami by a modification of the guanidine thiocyanate method (Chirgwin, 1979). Poly A<sup>+</sup> RNA was purified using a  
10     FastTrack kit (Invitrogen Corp., San Diego, CA). Double stranded (ds) cDNA was synthesized from 4.6 µg of poly A<sup>+</sup> RNA according to Gubler and Hoffman (1983) with minor modifications. The resulting cDNA was ligated to  
15     BstXI/EcoRI adaptors (Invitrogen Corp.) and the excess adaptors removed by exclusion column chromatography. High molecular weight fractions of size-selected ds-cDNA were ligated in pEXJ.T7 (an Okayama and Berg expression vector modified from pcEXV (Miller & Germain, 1986) to contain BstXI and other additional restriction sites and  
20     a T7 promoter (Stratagene) and electroporated in E.coli MC 1061 (Gene Pulser, Biorad). A total of  $3 \times 10^6$  independent clones with a mean insert size of 2.2 kb were generated. The library was plated on agar plates (Ampicillin selection) in 584 primary pools of ~5,000  
25     independent clones. After 18 hours amplification, the bacteria from each pool were scraped, resuspended in 4 mL of LB media and 0.75 mL processed for plasmid purification (QIAwell-96 ultra, Qiagen, Inc., Chatsworth, CA). Aliquots of each bacterial pool were stored at -85°C  
30     in 20% glycerol.

To screen the library, COS-7 cells were plated in slide chambers (Lab-Tek) in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf serum, 100 U/mL of  
35     penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine (DMEM-C) and grown at 37°C. in a humidified 5% CO<sub>2</sub> atmosphere for 24 hours before transfection. Cells were

transfected with minipr p DNA prepared from the primary pools (~4,500 cfu/pool) of the rat hypothalamus cDNA library using a modification of the DEAE-d xtran method (Warden & Thorne, 1968). Pools containing GALR1 were identified by PCR prior to screening and were omitted from the primary screen. The galanin binding assay was carried out after 48 hours. Cells were rinsed twice with phosphate-buffered saline (PBS) then incubated with 1 nM <sup>125</sup>I-porcine galanin (NEN; specific activity ~2200 Ci/mmol) in 20mM HEPES-NaOH, pH 7.4, containing 1.26 mM CaCl<sub>2</sub>, 0.81 mM MgSO<sub>4</sub>, 0.44 mM KH<sub>2</sub>PO<sub>4</sub>, 5.4 mM KCl, 10 mM NaCl, 0.1% BSA, and 0.1% bacitracin for one hour at room temperature. After rinsing and fixation in 2.5% glutaraldehyde, slides were rinsed in PBS, air-dried, and dipped in photoemulsion (Kodak, NTB-2). After a 3-4 day exposure slides were developed in Kodak D19 developer, fixed, and coverslipped (Aqua-Mount, Lerner Laboratories), then inspected for positive cells by brightfield microscopy (Leitz Laborlux, 25X magnification). One pool with positive cells, (J126) was subdivided and rescreened repeatedly until a single colony was isolated that conferred galanin binding. The 3.8 kb cDNA is preferably sequenced on both strands using Sequenase (US Biochemical, Cleveland, OH) according to the manufacturer. Nucleotide and peptide sequence analyses are performed using the Wisconsin Package (GCG, Genetics Computer group, Madison, WI) or PC/GENE (Intelligenetics, Mountain View, CA).

### 30 PCR Methodology

PCR reactions were carried out in 20μl volumes using Taq Polymerase (Boehringer Mannheim, Indianapolis, IN) in a buffer containing 10mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01% gelatin, 0.2 mM each dNTP, and 1 μM each PCR primer. To prescreen library pools for GALR1, two GALR1 prim r sets were used (KS-1177/1178 and KS-1311/1313, see below) to determine whether GALR1 was

present in original bacterial stocks of each library pool. PCR was carried out for 40 cycles of 94°C/2 min, 68°C/2 min, 72°C/3 min. Pools positive for GALR1 by PCR were eliminated from the library screen.

5

To confirm that the purified cDNA conferring galanin binding was distinct from GALR1, the isolated clone representing pool J126-10-334 (K985) was subjected to PCR analysis using three GALR1 primer sets representing different regions of GALR1. The nucleotide sequences of the primer sets are shown below:

10

15

KS-1177: 5'-TGG GCA ACA GCC TAG TGA TCA CCG -3' (Seq. I.D. No. 1) Nucleotides 146-169 of human GALR1 coding region, forward primer.

20

KS-1178: 5'-CTG CTC CCA GCA GAA GGT CTG GTT -3' (Seq. I.D. No. 2) Nucleotides 547-570 of human GALR1 coding region, reverse primer.

25

KS-1311: 5'-CCT CAG TGA AGG GAA TGG GAG CGA -3' (Seq. I.D. No. 3) Nucleotides 21-44 of rat GALR1 coding region, forward primer.

30

KS-1313: 5'-CTC ATT GCA AAC ACG GCA CTT GAA CA -3' (Seq. I.D. No. 4) Nucleotides 944-969 of rat GALR1 coding region, reverse primer.

35

KS-1447: 5'-CTT GCT TGT ACG CCT TCC GGA AGT -3' (Seq. I.D. No. 5) Nucleotides 920-943 of rat GALR1 coding region, reverse primer.

KS-1448: 5'-GAG AAC TTC ATC ACG CTG GTG GTG -3' (Seq. I.D. No. 6). Nucleotides 91-114 of rat GALR1 coding region, forward primer.

#### Generation of Human GALR2 PCR product

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Human genomic DNA (1  $\mu$ g; 12 different lots from Promega and Clontech) were amplified in 50  $\mu$ l PCR reaction mixtures using the Expand Long Template PCR System (as supplied and described by the manufacturer, Boehringer Mannheim) and 1  $\mu$ M of primers, using a program consisting of 40 cycles of 94°C for 2 min, 60°C for 2 min, and 68°C for 3 min, with a pre- and post-incubation of 95°C for 5 min and 68°C for 10 min, respectively. PCR primers for hGALR2 were designed against rGALR2 sequence: forward primer NS525 in the fourth transmembrane domain, and reverse primer NS526 in the sixth transmembrane domain. The PCR products were run on a 0.8% low-melting agarose gel. The single  $\approx$ 300 bp fragment from 3 different lots were isolated, purified by phenol extraction and subjected to sequencing using the T7 Sequenase PCR product sequencing kit (Amersham). Sequence was analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI).

#### 20 5' and 3' RACE Analysis of Human GALR2

5' and 3' RACE (rapid analysis of cDNA ends) were performed on human brain and human lung RNAs (Clontech), respectively, using a Marathon cDNA Amplification Kit (Clontech). Total RNA was poly A+ selected using a FastTrack mRNA Isolation Kit (Invitrogen Corp., San Diego, CA). For 5' RACE, double stranded (ds) cDNA was synthesized from 1  $\mu$ g Poly A+ RNA using BB 153, a reverse primer from the 5' end of the sixth transmembrane domain of hGALR2 from the PCR fragment described above. Adaptor ligation and nested PCR were performed according to the Marathon cDNA Amplification protocol using Advantage KlenTaq Polymerase (Clontech). The initial PCR reaction was performed on 1  $\mu$ l of a 50 fold dilution of the ligated cDNA using the supplier's Adaptor Primer 1 and BB 154, a reverse primer from the fifth transmembrane domain of th hGALR2 PCR product above. One  $\mu$ l of this initial PCR reaction was re-

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amplified using the Adaptor Primer 2 and NS 563, a reverse primer just upstream from BB154. The conditions for PCR were 30 sec at 94°C, 4 min at 72°C for 5 cycles, 30 sec at 94°C, 4 min at 70°C for 5 cycles, 20 sec at 94°C, 4 min at 68°C for 25 cycles, with a pre- and post-incubation of 1 min at 94°C and 7 min at 68°C respectively. A 600 base pair fragment from the nested PCR was isolated from a 1% TAE gel using a GENECLAN III kit (BIO 101, Vista, CA) and sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). The sequence was run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI). For 3' RACE, double stranded (ds) cDNA was synthesized from 1 µg Poly A+ RNA using the cDNA synthesis primer CDS supplied with the Marathon cDNA Amplification Kit (Clontech). PCR conditions for 3' RACE were similar to 5' RACE except that BB166 and BB167, forward primers from the fifth transmembrane domain of the hGALR2 PCR fragment described above, were used in place of BB154 and NS563, respectively. A 500 base pair fragment from the nested PCR was isolated from a 1% TAE gel using a GENECLAN III kit (BIO 101, Vista, CA) and sequenced as above.

#### Construction and Screening of a Human Heart cDNA Library

Poly A+ RNA was purified from human heart RNA (Clontech) using a FastTrack kit (Invitrogen, Corp.). DS- cDNA was synthesized from 8 µg of poly A+ RNA according to Gubler and Hoffman (1983) with minor modifications. The resulting cDNA was ligated to BstXI adaptors (Invitrogen, Corp.) and the excess adaptors removed by exclusion column chromatography. High molecular weight fractions of size-selected ds-cDNA were ligated in pEXJ.BS, an Okayama and Berg expression vector modified from pEXV (Miller and Germain, 1986) to contain BstXI and other additional restriction sites. A total of  $4.45 \times 10^6$  independent clones with a mean insert size of 2.5 kb were

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generated. The library was plated on agar plates (Ampicillin selection) in 127 primary pools; 50 pools with 37,500 independent clones, 51 pools with 25,000 clones and 26 pools with 50,000 clones. Glycerol stocks of the primary pools were combined in 16 superpools of 8 and screened for hGLR2 by PCR using primers BB153 and BB169, a forward primer from the second intracellular domain of hGALR2 identified in the 5' RACE fragment above. PCR was performed with the Expand Long Template PCR System (Boehringer Mannheim) under the following conditions: 1 min at 94°C, 4 min at 68°C for 40 cycles, with a pre- and post-incubation of 5 min at 95°C and 7 min at 68°C, respectively. Primary pools from positive superpools were screened by PCR and then primary pool 169 was subdivided and screened by PCR. One positive subpool, 69-11, was subdivided into 20 pools of 1200 clones plated on agar plates (ampicillin selection). Colonies were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH), denatured in 0.4 N NaOH, 1.5 M NaCl, renatured in 1M Tris, 1.5 M NaCl, and UV cross-linked. Filters were hybridized overnight at 40°C in a buffer containing 50 % formamide, 5X SSC, 7 mM TRIS, 1X Denhardt's solution and 25 µg/ml salmon sperm DNA (Sigma Chemical Co.) and 10<sup>6</sup> cpm/ml of KS1567, an oligonucleotide probe from the 3' end of the fifth transmembrane domain of hGALR2, labeled with γ-<sup>32</sup>P[ATP] (6000Ci/mmol, NEN) using polynucleotide kinase (Boehringer Mannheim). Filters were washed 2 x 15 minutes at room temperature in 2XSSC, 0.1% SDS, 2 x 15 minutes at 50°C in 0.1XSSC, 0.1% SDS, and exposed to XAR X-ray film (Kodak) for 3 days. Colonies which appeared to hybridize were re-screened by PCR using primers BB167 and BB170, a reverse primer from the COOH terminus of hGLR2 identified by the 3' RACE fragment above. PCR was performed with the Expand Long Template PCR System (Boehringer Mannheim) under the following conditions: 1 min at 94°C, 2 min at 58°C, 2 min at 68°C for 28 cycles,

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with a pre- and post-incubation of 5 min at 95°C and 7 min at 68°C respectively. One positive colony, 69-11-5 was amplified overnight in 10 ml LB media and processed for plasmid purification using a standard alkaline lysis miniprep procedure followed by a PEG precipitation. To ensure that 69-11-5 was a single colony, it was amplified for 3 hours in 3 ml of LB media and then 1 µl of a 1:100 dilution was plated on an agar plate. Twenty colonies were screened by PCR using primers BB167 and BB170 using the same conditions as above, except that 25 cycles were used instead of 28. One positive single colony, 69-11-5-3, designated B029, was amplified overnight in 10 ml of TB media and processed for plasmid purification. Vector-anchored PCR was performed on B029 using the Expand Long Template PCR System (Boehringer Mannheim) to determine the orientation and size of the insert. BB173 and BB172, forward and reverse vector primers, respectively, were used with primers BB169 and BB153. The conditions for PCR were 1 min at 94°C, 4 min at 68°C for 36 cycles, with a pre- and post-incubation of 5 min at 95°C and 7 min at 68°C respectively. B029 is preferably sequenced on both strands using AmpliTaq DNA Polymerase, FS (Perkin Elmer). The sequence is run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, WI).

To test the ability of 69-11-5 to confer galanin binding, COS-7 cells were plated in slide chambers (Lab-Tek) in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf serum, 100U/ml of penicillin, 100 µg/ml streptomycin, 2mM L-glutamine (DMEM-c) and grown at 37°C in a humidified 5% CO<sub>2</sub> atmosphere for 24 hours before transfection. Cells were transfected with 1 µg of miniprep DNA from 69-11-5 or vector control using a modification of the DEAE-dextran method (Warden and Thorne, 1968). 48 hours after transfection, cells were rinsed with phosphate-buffered saline (PBS) then

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incubated with 1 nM  $^{125}\text{I}$ -rat galanin (NEN; specific activity ~2200 Ci/mmol) and 2 nM  $^{125}\text{I}$ -porcine galanin (NEN; specific activity ~2200 Ci/mmol) in 20mM HEPES-NaOH, pH 7.4, containing 1.26 mM  $\text{CaCl}_2$ , 0.81 mM  $\text{MgSO}_4$ , 0.44 mM  $\text{KH}_2\text{PO}_4$ , 5.4 mM KCl, 10 mM NaCl, 0.1% BSA, and 0.1% bacitracin for one hour at room temperature. After rinsing and fixation in 2.5% glutaraldehyde, slides were rinsed in PBS, air-dried, and dipped in photoemulsion (Kodak, NTB-2). After a 4-day exposure, slides were developed in Kodak D19 developer, fixed, and coverslipped (Aqua-Mount, Lerner Laboratories), then inspected for positive cells by brightfield microscopy (Leitz Laborlux, 25X magnification). To test the ability of the single clone B029 to confer galanin binding, B029 or control vector were transfected into COS-7 cells for testing of  $^{125}\text{I}$  galanin as described above, with the exception that after fixation, binding of  $^{125}\text{I}$  galanin to cells on the slide was detected using an  $^{125}\text{I}$  probe (Mini-Instruments, Ltd., Essex, England). The signal from B029 transfected cells was compared with the signal from control vector transfected cells.

#### Primers and probes used

NS525: 5'CCCTACCTGAGCTACTACCGTCA 3' (SEQ ID NO:15);

NS526: 5'ACCAAACCACACGCAGAGGATAAG 3' (SEQ ID NO:16);

BB153: 5'-CCACGATGAGGATCATGCGTGTCCACC-3' (SEQ ID NO:17);

BB154: 5'-TAGGTCAGGCCGAGAACCAGCACAGG-3' (SEQ ID NO:18);

NS563: 5'-CAGGTAGCTGAAGACGAAGGTGCA-3' (SEQ ID NO:19);

BB166: 5'-CTGCACCTTCGTCTTCAGCTACCTG-3' (SEQ ID NO:20);

BB167: 5'-CCTGTGCTGGTTCTCGGCCTGACCTA-3' (SEQ ID NO:21);



BB169: 5'-TATCTGGCCATCCGCTACCCGCTGCA-3' (SEQ ID NO:22);

KS1567: 5'-TTGCGCTACCTCTGGCGCGCCGTCGACCCGGTGGCCGCGGGCTCG-  
3' (SEQ ID NO:23);

5

BB170: 5'-CCAACAATGACTCCAACCTCTGTGAC-3' (SEQ ID NO:24);

BB173: 5'-AGGCGCAGAACTGGTAGGTATGGAA-3' (SEQ ID NO:25);

10

and

BB172: 5'-AAGCTTCTAGAGATCCCTCGACCTC-3' (SEQ ID NO:26).

#### Generation of an Intronless Human GALR2 Receptor

15

Human tissues may be screened by PCR, using primers that cross the intron, to identify cDNA sources that express the intronless form. An intronless hGALR2 clone may be obtained using an approach similar to that used to obtain an intronless rGALR2 clone (*infra*). Alternatively, one

20

may use restriction enzymes to remove the intron and some adjacent coding region from B029, and then replace the removed coding region by inserting a restriction enzyme-digested PCR fragment amplified from a tissue shown to express the intronless form of the receptor.

25

Human hippocampus and human hypothalamus were each shown to express the intronless form. A full-length, intronless human GALR2 PCR product was amplified from human hippocampus, but was found to contain a single point mutation downstream from the intron splice site. Therefore, an EcoRI/StyI restriction digest fragment, containing 11 bp of 5'UT and the first 557 bp of hGalR2 coding region, was ligated to a StyI restriction digest fragment, containing bp 558-1164 of the coding region and

30

182 bp of 3' UT, which was isolated from the intron-containing hGalR2 clon (B029). The ligation product,

35

comprising the entire intronless form of th human GALR2

receptor, was subcloned into the vector pEXJ and designated B039.

#### Northern Blots

5 Human brain multiple tissue northern blots (MTN blots II and III, Clontech, Palo Alto, CA) carrying mRNA purified from various human brain areas may be hybridized according to the manufacturers' specifications.

10 Rat multiple tissue northern blots including multiple brain tissue blots (rat MTN blot, Clontech, Palo Alto, CA) carrying mRNA purified from various rat tissues also may be hybridized at high stringency according to the manufacturer's specifications.

15

#### RT-PCR analyses of GALR2 mRNA

Tissues may be homogenized and total RNA extracted using the guanidine isothiocyanate/CsCl cushion method. RNA may then be treated with DNase to remove any  
20 contaminating genomic DNA. cDNA may be prepared from total RNA with random hexanucleotide primers using the reverse transcriptase Superscript II (BRL, Gaithersburg, MD). First strand cDNA (about 250 ng of total RNA) may be amplified for example, in a 50  $\mu$ L PCR reaction mixture  
25 (200  $\mu$ M dNTPs final concentration) and 1  $\mu$ M appropriate primers, using an appropriate thermal cycling program.

The PCR products may be run on a 1.5% agarose gel and transferred to charged nylon membranes (Zetaprobe GT, BioRad), and analyzed as Southern blots. GALR2 primers  
30 will be screened for the absence of cross-reactivity with the other galanin receptors. Filters may be hybridized with radiolabeled probes and washed under high stringency. Labeled PCR products may be visualized on X-ray film. Similar PCR and Southern blot analyses may be  
35 conducted with primers and probes, e.g., 1B15, direct d to the housekeeping gene, glyceraldehyde phosphate

dehydrogenase (Clontech, Palo Alto, CA), to normalize the amount of cDNA used from the different tissues.

5 RT-PCR of rat brain tissues was carried out using total or poly A<sup>+</sup> RNA (1.5 µg or 0.5 µg, respectively) isolated from various rat brain regions and converted to cDNA using Superscript II (BRL, Gaithersburg, MD) reverse transcriptase with random priming. The cDNAs were used as templates for PCR amplification of GALR2 using  
10 specific GALR2 primers. PCR products were separated on an agarose gel by electrophoresis and blotted to a charged nylon membrane.

#### Isolation of the Intronless Rat GALR2

15 RT-PCR analysis of various rat brain regions (Figure 5) was carried out using primers representing N- and C-termini of rat GALR2 (supra). The forward and reverse primers comprised nucleotides 1-23 and 1087-1110, respectively, of the intronless rat GALR2 sequence (SEQ.  
20 ID No. 7). The PCR products were separated by agarose gel electrophoresis, blotted, and hybridized with an oligonucleotide probe designed to the predicted 5/6 loop of GALR2 (nucleotides 651-695, SEQ. ID No. 7). This analysis indicated the presence of both intron-containing  
25 and intronless forms of rat GALR2 in brain. In order to choose an appropriate tissue source from which to isolate the intronless form, a similar PCR analysis on RNA from a variety of rat tissues was carried out. Based on the size of the products determined by agarose gel  
30 electrophoresis (data not shown), rat heart was chosen as a potential source of intronless GALR2 RNA. To isolate the intronless GALR2, PCR primers similar to those used above but containing restriction enzyme sites to facilitate subcloning and a Kozak consensus for  
35 translation initiation (KS-1550 and KS-1551, see below) were used to amplify rat GALR2 from rat heart RNA by PCR (after conversion of the RNA to first strand cDNA by

standard methods). A PCR product of the correct size was isolated from an agarose gel and then reamplified using the same primers to increase yield. The products were digested with the appropriate restriction enzymes to produce cohesive ends (EcoRI and Xba I), ligated into the expression vector EXJ.RH and transformed into E.coli. The resulting colonies were transferred to nitrocellulose membranes and hybridized with an oligonucleotide probe to the predicted 2/3 loop of rat GALR2 (nucleotides 259-303, SEQ. ID No. 7). A single hybridizing colony was found by subsequent analysis to contain the intronless rat GALR2 cDNA.

Primers used:

Forward primer, KS-1550: 5'-ACGGAATTCGACATGAATGGCTCCGGCA (SEQ. ID No. 27)

Reverse Primer, KS-1551:

5'-GCTCTAGAGCCCCTTTGGTCCTTTAACAAGCCGG (SEQ. ID No. 28)

Production of Recombinant Baculovirus

The coding region of GALR2 may be subcloned into pBlueBacIII into existing restriction sites, or sites engineered into sequences 5' and 3' to the coding region of GALR2, for example, a 5' BamHI site and a 3' EcoRI site. To generate baculovirus, 0.5 µg of viral DNA (BaculoGold) and 3 µg of GALR2 construct may be co-transfected into  $2 \times 10^6$  Spodoptera frugiperda insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined in by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells then are incubated for 5 days at 27°C.

The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus

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st cks are as described in Pharmingen's manual.

### Cell Culture

5 COS-7 cells are grown on 150 mm plates in DMEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37°C, 5% CO<sub>2</sub>. Stock plates of COS-7 cells are trypsinized and split 1:6 every 3-4 days. Human embryonic kidney 293 cells are grown on 10 150 mm plates in D-MEM with supplements (minimal essential medium) with Hanks' salts and supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37°C, 5% CO<sub>2</sub>. Stock plates of 293 cells 15 are trypsinized and split 1:6 every 3-4 days. Mouse fibroblast LM(tk-) cells are grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37°C, 5% CO<sub>2</sub>. Stock 20 plates of LM(tk-) cells are trypsinized and split 1:10 every 3-4 days. Chinese hamster ovary (CHO) cells were grown on 150 mm plates in HAM's F-12 medium with supplements (10% bovine calf serum, 4 mM L-glutamine and 100 units/mL penicillin/100 ug/ml streptomycin) at 37°C, 25 5% CO<sub>2</sub>. Stock plates of CHO cells were trypsinized and split 1:8 every 3-4 days.

LM(tk-) cells stably transfected with the GALR2 receptor may be routinely converted from an adherent monolayer to 30 a viable suspension. Adherent cells are harvested with trypsin at the point of confluence, resuspended in a minimal volume of complete DMEM for a cell count, and further diluted to a concentration of 10<sup>6</sup> cells/mL in suspension media (10% bovine calf serum, 10% 10X Medium 35 199 (Gibco), 9 mM NaHCO<sub>3</sub>, 25 mM glucose, 2 mM L-glutamine, 100 units/mL penicillin/100 µg/mL streptomycin, and 0.05% methyl cellulose). Cell suspensions are maintained in a

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shaking incubator at 37°C, 5% CO<sub>2</sub> for 24 hours. Membranes harvested from cells grown in this manner may be stored as large, uniform batches in liquid nitrogen. Alternatively, cells may be returned to adherent cell culture in complete DMEM by distribution into 96-well microtiter plates coated with poly-D-lysine (0.01 mg/mL) followed by incubation at 37°C, 5% CO<sub>2</sub> for 24 hours. Cells prepared in this manner generally yield a robust and reliable response in cAMP radio-immunoassays as further described hereinbelow.

Mouse embryonic fibroblast NIH-3T3 cells are grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 µg/mL streptomycin) at 37°C, 5% CO<sub>2</sub>. Stock plates of NIH-3T3 cells are trypsinized and split 1:15 every 3-4 days.

Sf9 and Sf21 cells are grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27°C, no CO<sub>2</sub>. High Five insect cells are grown on 150 mm tissue culture dishes in Excell 400™ medium supplemented with L-Glutamine, also at 27°C, no CO<sub>2</sub>.

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#### Transfection

All receptor subtypes studied may be transiently transfected into COS-7 cells by the DEAE-dextran method, using 1 µg of DNA /10<sup>6</sup> cells (Cullen, 1987). In addition, Schneider 2 Drosophila cells may be cotransfected with vectors containing the receptor gene, under control of a promoter which is active in insect cells, and a selectable resistance gene, eg., the G418 resistant neomycin gene, for expression of the galanin receptor.

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#### Stable Transfection

The GALR2 receptor may be co-transfected with a G-418

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r sistant gene into th human embryonic kidney 293 cell line by a calcium phosphate transfection method (Cullen, 1987). GALR1 receptors were expressed in cells using methods well-known in the art. Stably transfected cells are selected with G-418. GALR2 receptors may be similarly transfected into mouse fibroblast LM(tk-) cells, Chinese hamster ovary (CHO) cells and NIH-3T3 cells. Transfection of LM(tk-) cells with the plasmid K985 and subsequent selection with G-418 resulted in the LM(tk-) cell line L-rGALR2-8 (ATCC Accession No. CRL-12074), which stably expresses the rat GALR2 receptor. A similar procedure was used to transfect LM(tk-) cells with plasmid K1045 (intronless rat GALR2 receptor construct) resulting in the LM(tk-) cell line L-rGALR4-I (ATCC Accession No. CRL-12223). In addition, this procedure was used to transfect CHO cells with an intron-containing plasmid to create a stably expressing rat GALR2 CHO cell line, C-GalR2-79 (ATCC Accession No. \_\_\_\_\_).

#### 20 Radioligand binding assays

Transfected cells from culture flasks were scraped into 5 ml of Tris-HCl, 5mM EDTA, pH 7.5, and lysed by sonication. The cell lysates were centrifuged at 1000 rpm for 5 min. at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min. at 4°C. The pellet was suspended in binding buffer (50mM Tris-HCl, 5mM MgSO<sub>4</sub>, 1mM EDTA at pH 7.5 supplemented with 0.1% BSA, 2µg/ml aprotinin, 0.5mg/ml leupeptin, and 10µg/ml phosphoramidon). Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10% of the added radioligand, were added to 96-well polpropylene microtiter plates containing <sup>125</sup>I-labeled peptide, non-labeled peptides and binding buffer to a final volume of 250 µl. In equilibrium saturation binding assays membrane preparations were incubated in the presence of increasing concentrations (0.1nM to 4nM) of [<sup>125</sup>I]porcine galanin (specific activity 2200 Ci/mmol).

The binding affinities of the different galanin analogs were determined in equilibrium competition binding assays, using 0.1 nM [<sup>125</sup>I]porcin galanin in the presence of twelve different concentrations of the displacing ligands. Binding reaction mixtures were incubated for 1 hr at 30°C, and the reaction was stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity was measured by scintillation counting and data were analyzed by a computerized non-linear regression program. Non-specific binding was defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100nM of unlabeled porcine galanin. Protein concentration was measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard.

Binding assays involving the rat GALR3 receptor are conducted at room temperature for 120 min. in binding buffer. Leupeptin, aprotonin and phosphoramidon are omitted from rat GALR3 assays while bacitracin is added to 0.1%. Nonspecific binding is defined in the presence of 1 μM porcine galanin. Cells transiently or stably expressing GALR3 receptors are produced using transfection methods which are well-known in the art, examples of which are provided herein (supra). The rat GALR3 receptor may be expressed using plasmid K1086, deposited on October 8, 1996, with the ATCC, 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and was accorded ATCC Accession No. 97747. Another plasmid expressing the rat GALR3 receptor is plasmid pEXJ-rGALR3t, deposited with the ATCC under the Budapest Treaty on December 17, 1996, and accorded ATCC Accession No. 97826. The human GALR3 receptor may be expressed using plasmid pEXJ-hGALR3, also deposited with the ATCC under the Budapest Treaty on December 17, 1996,



and accorded ATCC Accession No. 97827. Cells stably expressing the GALR3 receptors may be used in functional assays well known in the art, examples of which are provided herein (infra).

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### Functional Assays

#### Cyclic AMP (cAMP) formation

The receptor-mediated inhibition of cyclic AMP (cAMP) formation may be assayed in LM(tk-) cells expressing the rat GALR1 and GALR2 receptors. Cells were plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5mM theophylline, 2 $\mu$ g/ml aprotinin, 0.5mg/ml leupeptin, and 10 $\mu$ g/ml phosphoramidon for 20 min at 37°C, in 5% CO<sub>2</sub>. Galanin or the test compounds were added and incubated for an additional 10 min at 37°C. The medium was aspirated and the reaction was stopped by the addition of 100 mM HCl. The plates were stored at 4°C for 15 min, and the cAMP content in the stopping solution was measured by radioimmunoassay. Radioactivity was quantified using a gamma counter equipped with data reduction software.

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Functional assay experiments were also performed using stably transfected cells seeded into 96-well microtiter plates and cultured until confluent. To reduce the potential for receptor desensitization, the serum component of the media was reduced to 1.5% for 4 to 16 hours before the assay. Cells were washed in Hank's buffered saline, or HBS (150 mM NaCl, 20 mM HEPES, 1 mM CaCl<sub>2</sub>, 5 mM KCl, 1 mM MgCl<sub>2</sub>, and 10 mM glucose) supplemented with 0.1% bovine serum albumin plus 5 mM theophylline and pre-equilibrated in the same solution for 20 min at 37°C in 5% CO<sub>2</sub>. Cells were then incubated 5 min with 10  $\mu$ M forskolin and various concentrations of receptor-selective ligands. The assay was terminated by the removal of HBS and acidification of the cells with

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100 mM HCl. Intracellular cAMP was extracted and quantified with a modified version of a magnetic bead-based radioimmunoassay (Advanced Magnetics, Cambridge, MA). The final antigen/antibody complex was separated from free  $^{125}\text{I}$ -cAMP by vacuum filtration through a PVDF filter in a microtiter plate (Millipore, Bedford, MA). Filters were punched and counted for  $^{125}\text{I}$  in a Packard gamma counter. Functional studies of the rat GALR1 receptor in LMTK- cells were performed as previously described above except that leupeptin, aprotinin and phosphoramidon were omitted from the assay, and cells were stimulated with forskolin plus peptides for a period of 5 min.

#### 15 Arachidonic Acid Release

CHO cells stably transfected with the rat GALR2 receptor were seeded into 96 well plates and grown for 3 days in HAM's F-12 with supplements.  $^3\text{H}$ -arachidonic acid (specific activity = 0.75 uCi/ml) was delivered as a 100 ul aliquot to each well and samples were incubated at 37°C, 5%  $\text{CO}_2$  for 18 hours. The labeled cells were washed three times with 200 ul HAM's F-12. The wells were then filled with medium (200 uL) and the assay was initiated with the addition of peptides or buffer (22 uL). Cells were incubated for 30 min at 37°C, 5%  $\text{CO}_2$ . Supernatants were transferred to a microtiter plate and evaporated to dryness at 75°C in a vacuum oven. Samples were then dissolved and resuspended in 25 uL distilled water. Scintillant (300 uL) was added to each well and samples were counted for  $^3\text{H}$  in a Trilux plate reader. Data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

#### 35 Intracellular calcium mobilization

The intracellular free calcium concentration may be measured by microspectrofluorometry using the fluorescent

indicator dye Fura-2/AM (Bush et al. 1991). Cells stably transfected with GALR2 are seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells are washed with HBS and loaded with 100  $\mu$ L of Fura-2/AM (10  $\mu$ M) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10 to 20 min. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission is determined at 510 nm with excitation wavelengths alternating between 340 nm and 380 nm. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

#### Phosphoinositide metabolism

LM(tk-) cells stably expressing the rat GALR2 receptor cDNA were plated in 96-well plates and grown to confluence. The day before the assay the growth medium was changed to 100  $\mu$ L of medium containing 1% serum and 0.5  $\mu$ Ci [ $^3$ H]myo-inositol, and the plates were incubated overnight in a CO<sub>2</sub> incubator (5% CO at 37°C). Alternatively, arachidonic acid release may be measured if [ $^3$ H]arachidonic acid is substituted for the [ $^3$ H]myo-inositol. Immediately before the assay, the medium was removed and replaced by 200  $\mu$ L of PBS containing 10 mM LiCl, and the cells were equilibrated with the new medium for 20 min. During this interval cells were also equilibrated with the antagonist, added as a 10  $\mu$ L aliquot of a 20-fold concentrated solution in PBS. The [ $^3$ H]inositol-phosphates accumulation from inositol phospholipid metabolism was started by adding 10  $\mu$ L of a solution containing the agonist. To the first well 10  $\mu$ L were added to measure basal accumulation, and 11 different concentrations of agonist were assayed in the following 11 wells of each plate row. All assays were performed in duplicate by repeating the same additions in two consecutive plate rows. The plates were incubated in

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a CO<sub>2</sub> incubator for 1 hr. The reaction was terminated by adding 15 µl of 50% v/v trichloroacetic acid (TCA), followed by a 40 min. incubation at 4°C. After neutralizing TCA with 40 µl of 1M Tris, the content of the wells was transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates were prepared adding 200 µL of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates were placed on a vacuum manifold to wash or elute the resin bed. Each well was washed 2 times with 200 µL of water, followed by 2 x 200 µL of 5mM sodium tetraborate/60 mM ammonium formate. The [<sup>3</sup>H]IPs were eluted into empty 96-well plates with 200 µl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells was added to 3 mls of scintillation cocktail, and the radioactivity was determined by liquid scintillation counting.

Functional assays using GALR3 receptors are performed similarly.

It is to be understood that the cell lines described herein are merely illustrative of the methods used to evaluate the binding and function of the galanin receptors of the present invention, and that other suitable cells may be used in the assays described herein.

#### Functional responses in oocytes expressing GalR2

Female Xenopus laevis (Xenopus-1, Ann Arbor, MI) were anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary was removed using aseptic technique (Quick and Lester, 1994). Oocytes were defolliculated using 2 mg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in a solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM MgCl<sub>2</sub>, and 5 mM HEPES, pH 7.5. Oocytes were injected (Nanoject,

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Drummond Scientific, Broomall, PA) with 50 nL of rat GalR2 mRNA or other mRNA for use as a negative control. RNA was prepared by linearization of the plasmid (pBluescript) containing the entire coding region of the GalR2 cDNA, followed by in vitro transcription using the T7 polymerase ("MessageMachine", Ambion). Alternatively, mRNA may be translated from a template generated by PCR, incorporating a T7 promoter. Oocytes were incubated at 16° on a rotating platform for 3-8 days post-injection. Dual electrode voltage clamp ("GeneClamp", Axon Instruments Inc., Foster City, CA) was performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes were clamped at a holding potential of -80 mV. During recordings, oocytes are bathed in continuously flowing (2-5 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 5 mM HEPES, pH 7.5 (ND96). Drugs are applied by switching from a series of gravity fed perfusion lines.

The human GALR2 receptor and GALR3 receptors may be studied functionally using similar methods.

#### Galanin Receptor Autoradiography

Male Sprague-Dawley rats (Charles River, Wilmington, Massachusetts) were euthanized using CO<sub>2</sub>, decapitated, and their brains immediately removed and frozen on dry ice. Tissue sections were cut at 20 µm using a cryostat and thaw mounted onto gelatin coated slides. Tissues were preincubated in two 10 minute changes of 50 mM Tris-HCl buffer pH 7.4, containing 5 mM MgSO<sub>4</sub> and 2 mM EGTA (Sigma). The radioligand binding was carried out in the same buffer, which also contained 0.1% bovine serum albumin, 0.02% aprotinin, 0.031% leupeptin, 0.1% phosphoramidate (Boehringer Mannheim), and 0.1 nM [<sup>125</sup>I]porcine galanin (specific activity 2200 Ci/mmol, NEN) for 1 hour at 22°C. Nonspecific binding was determined

in the presence of 5  $\mu$ M porcine galanin (Bachem). As [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> was shown to be selective for the cloned GALR2 receptor (*infra*), a 60 nM concentration of this peptide was used to displace [<sup>125</sup>I]galanin binding from the rat brain tissue sections. The use of this concentration was based on the binding data, which showed the affinity of [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> to be 6 nM at the GALR2 receptor, and 3  $\mu$ M at the GALR1 receptor. In general, a 10X concentration of the blocking ligand is sufficient to remove 100% of the targeted receptor, while leaving the GALR1 receptor unaffected. After incubation, tissues were dipped twice in ice-cold Tris-HCl buffer (4°C), followed by a 5 minute wash in ice-cold Tris-HCl buffer (4°C), then dipped twice in ice-cold deionized water to remove the salts. Sections were placed in X-ray cassettes and apposed to Dupont Cronex MRF 34 Film for 5 days. Films were developed using a Kodak M35A Processor.

#### Tissue preparation for neuroanatomical studies

Male Sprague-Dawley rats (Charles River) are decapitated and the brains rapidly removed and frozen in isopentane. Coronal sections are cut at 11  $\mu$ m on a cryostat and thaw-mounted onto poly-L-lysine coated slides and stored at -80°C until use. Prior to hybridization, tissues are fixed in 4% paraformaldehyde, treated with 5 mM dithiothreitol, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, delipidated with chloroform, and dehydrated in graded ethanols.

#### Probes

Oligonucleotide probes employed to characterize the distribution of the rat GALR2 receptor mRNA may be synthesized, for example, on a Millipore Expedite 8909 Nucleic Acid Synthesis System. The probes are then lyophilized, reconstituted in sterile water, and purified on a 12% polyacrylamide denaturing gel. The purified probes are again reconstituted to a concentration of 100

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ng/ $\mu$ L, and stored at  $-20^{\circ}\text{C}$ . Probe sequences may include DNA or RNA which is complementary to the mRNA which encodes the GALR2 receptor.

5 Localization of GALR2 mRNA: In situ hybridization

Animals

Timed-pregnant female Sprague-Dawley rats were purchased from Charles River. The day of birth for each litter was designated as postnatal day 0 (P0). Brains were removed from pups on P0, P3, P5, P8, P10, P15, P20, and P25. The brains from the mothers were also removed and used as the adult comparison. All brains were sectioned in the coronal plane at  $11\ \mu\text{m}$  and the sections thaw-mounted on to poly-L-lysine coated microscope slides. The sections were then used for in situ hybridization histochemistry as described below.

Tissue preparation

Prior to hybridization, tissues were fixed in 4% paraformaldehyde, treated with 5 mM dithiothreitol, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, delipidated with chloroform, and dehydrated in graded ethanols. The sections were prehybridized for one hour at  $40^{\circ}\text{C}$  in hybridization buffer, which consisted of 50% formamide, 4X sodium citrate buffer (1X SSC = 0.15 M NaCl and 0.015 M sodium citrate), 1X Denhardt's solution (0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin), 50 mM dithiothreitol, 0.5 mg/ml salmon sperm DNA, 0.5 mg/ml yeast tRNA, and 10% dextran sulfate.

In Situ Hybridization

32mer oligonucleotide probes complementary to nucleotides 261-292 of the GALR2 mRNA were synthesized, purified, and 3'-end labeled with  $^{35}\text{S}$ -dATP (1200 Ci/mmol, New England Nuclear, Boston, MA) to a specific activity of  $10^9$  dpm/ $\mu\text{g}$  using terminal deoxynucleotidyl transferase (Boehringer

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Mannheim; Indianapolis, IN). The radiolabeled probes were purified on Biospin 6 chromatography columns (Bio-Rad; Richmond, CA), and diluted in the hybridization buffer described above to a concentration of  $1.5 \times 10^4$  cpm/ $\mu$ l. One hundred  $\mu$ l of the radiolabeled probe was applied to each section, which was then covered with a Parafilm coverslip. Hybridization was carried out overnight in humid chambers at 40 to 55°C. The following day the sections were washed in two changes of 2X SSC for one hour at room temperature, in 2X SSC for 30 min at 50-60°C, and finally in 0.1X SSC for 30 min at room temperature. Tissues were dehydrated in graded ethanols and apposed to Kodak XAR-5 film for 2 weeks at -20°C, then dipped in Kodak NTB3 autoradiography emulsion diluted 1:1 with 0.2% glycerol water. After exposure at 4°C for 4 weeks, the slides were developed in Kodak D-19 developer, fixed, and counterstained with hematoxylin and eosin.

#### Localization of GALR2 mRNA: Ribonuclease protection assay (RPA)

##### Development of probes

A cDNA fragment encoding a 467 BP fragment of the rGAL R2 was subcloned into a pBluescript plasmid vector. This construct was linearized with Xba I or Sal I. T3 and T7 RNA polymerases were used to synthesize the sense and antisense strands of RNA respectively. Full-length RNA transcripts were obtained using a full-length cDNA construct in the same vector.

A probe coding for rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, a constitutively expressed protein, was used concurrently. GAPDH is expressed at a relatively constant level in most tissue and its detection was used to compare expression levels of the rGalR2 gene in different tissues.

##### RNA Extraction



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RNA was isolated from rat peripheral tissue as well as regions of the CNS using a LiCl precipitation protocol (Cathala et al., 1983). Tissue was homogenized in 5M guanidine isothiocyanate, 50mM TRIS, 10 mM EDTA, using 7 ml of lysis buffer/gram tissue. 4M LiCl were added (7ml/ml homogenate) and the mixture were stored at 4°C for 24-48 hours. Homogenates were centrifuged and the pellets were resuspended in 3M LiCl, and centrifuged again. The pellets were resuspended in 0.1% sodium dodecyl sulfate (SDS), extracted in phenol:chloroform:isoamyl alcohol (24:24:1) and the RNA ethanol precipitated. Yield and relative purity were assessed by measuring absorbance  $A_{260}/A_{280}$ .

#### 15 Synthesis of probes

rGALR2 and GAPDH cDNA sequences preceded by phage polymerase promoter sequences were used to synthesize radiolabeled riboprobes. Conditions for the synthesis of riboprobes were: 1-2  $\mu$ l linearized template ( $1\mu$ g/ $\mu$ l), 20  $\mu$ l of ATP, GTP, UTP (10 mM each), 2  $\mu$ l dithiothreitol (0.1 M), 20 units RNasin RNase inhibitor, 1-2  $\mu$ l (15-20 units/ $\mu$ l) RNA polymerase, 4  $\mu$ l transcription buffer (Promega Corp.), and 5  $\mu$ l  $\alpha^{32}$ P-CTP (specific activity 800Ci/mmol). 0.1 mM CTP (0.02-1.0  $\mu$ l) were added to the reactions, and the volume were adjusted to 20  $\mu$ l with DEPC-treated water. Labeling reactions were incubated at 38°C for 90 min, after which 2 units of RQ1 RNase-free DNase (Promega Corp.) were added to digest the template. The riboprobes were separated from unincorporated nucleotide by a spun G-50 column (Select D G-50(RF); 5 Prime-3 Prime, Inc.). TCA precipitation and liquid scintillation spectrometry were used to measure the amount of label incorporated into the probe. A fraction of all riboprobes synthesized were size-fractionated on 0.4 mm thick 5% acrylamide sequencing gels and autoradiographed to confirm that the probes synthesized were full-length and not degraded.

Solution hybridization/ribonuclease protection assay

For solution hybridization 2-15 µg of total RNA isolated from tissues were used. Sense RNA synthesized using the full-length coding sequence of the rGalR2 was used to characterize specific hybridization. Negative controls consisted of 30 µg transfer RNA (tRNA) or no tissue blanks. All samples were placed in 1.5-ml microfuge tubes and vacuum dried. Hybridization buffer (40 µl of 400 mM NaCl, 20 mM Tris, pH 6.4, 2 mM EDTA, in 80% formamide) containing  $0.25-1.0 \times 10^6$  counts of each probe were added to each tube. Samples were heated at 90°C for 15 min, after which the temperature were lowered to 45°C for hybridization.

After hybridization for 14-18 hr, the RNA/probe mixtures were digested with RNase A (Sigma) and RNase T1 (Bethesda Research Labs). A mixture of 2.0 µg RNase A and 1000 units of RNase T1 in a buffer containing 330 mM NaCl, 10 mM Tris (pH 8.0) and 5 mM EDTA (400 µl) was added to each sample and incubated for 90 min at room temperature. After digestion with RNases, 20 µl of 10% SDS and 50 µg proteinase K were added to each tube and incubated at 37°C for 15 min. Samples were then extracted with phenol/chloroform:isoamyl alcohol and precipitated in 2 volumes of ethanol for 1 hr at -70°C. tRNA was added to each tube (30 mg) as a carrier to facilitate precipitation. Following precipitation, samples were centrifuged, washed with cold 70% ethanol, and vacuum dried. Samples were dissolved in formamide loading buffer and size-fractionated on a urea/acrylamide sequencing gel (7.6 M urea, 6% acrylamide in Tris-borate-EDTA). Gels were dried and apposed to Kodak XAR-5 x-ray film.

In vivo methods

The effects of galanin, galanin derivatives, and related peptides and compounds were evaluated by

intracerebroventricular (i.c.v.) injection of the peptide or compound followed by measurement of food intake in the animal. Measurement of food intake was performed for 3 hours after injection, but other protocols may also be used. Saline was injected as a control, but it is understood that other vehicles may be required as controls for some peptides and compounds. In order to determine whether a compound is a GALR2 antagonist, food intake in rats may be stimulated by administration of (for example) the GALR2-selective peptide agonist [D-Trp<sub>2</sub>]-galanin<sub>(1-29)</sub> through an intracerebroventricular (i.c.v.) cannula. A preferred anatomic location for injection is the hypothalamus, in particular, the paraventricular nucleus. Methods of cannulation and food intake measurements are well-known in the art, as are i.c.v. modes of administration (Kyrkouli et al., 1990, Ogren et al., 1992). To determine whether a compound reduces [D-Trp<sub>2</sub>]-galanin<sub>(1-29)</sub> stimulated food intake, the compound may be administered either simultaneously with the peptide, or separately, either through cannula, or by subcutaneous, intramuscular, or intraperitoneal injection, or more preferably, orally.

#### Materials

Cell culture media and supplements are from Specialty Media (Lavallette, NJ). Cell culture plates (150 mm and 96-well microtiter) are from Corning (Corning, NY). Sf9, Sf21, and High Five insect cells, as well as the baculovirus transfer plasmid, pBlueBacIII™, are purchased from Invitrogen (San Diego, CA). TMN-FH insect medium complemented with 10% fetal calf serum, and the baculovirus DNA, BaculoGold™, is obtained from Pharmingen (San Diego, CA.). Ex-Cell 400™ medium with L-Glutamine is purchased from JRH Scientific. Polypropylene 96-well microtiter plates are from Co-star (Cambridge, MA). All radioligands are from New England Nuclear (Boston, MA).

Galanin and related peptide analogs were either from Bachm California (Torrance, CA), Peninsula (Blmont, CA); or were synthesized by custom order from Chiron Mimotopes Peptide Systems (San Diego, CA).

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Bio-Rad Reagent was from Bio-Rad (Hercules, CA). Bovine serum albumin (ultra-fat free, A-7511) was from Sigma (St. Louis. MO). All other materials were reagent grade.

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## Experimental Results

### Isolation of a GALR2 cDNA from rat hypothalamus

In order to clone additional members of the galanin receptor family, an expression cloning strategy based on the potential presence of multiple galanin receptors in hypothalamus was designed. Although recent evidence indicated that GALR1 receptor mRNA was present in rat hypothalamus (Gustafson et al., 1996; Parker et al., 1995), not all aspects of the cloned GALR1 pharmacological profile match that observed for galanin-mediated feeding (Crawley et al., 1993). These results suggested that the regulation of galanin-induced feeding may not be explained by the presence of only GALR1 in the rat hypothalamus.

A randomly-primed cDNA expression library was constructed from rat hypothalamus and screened by radioligand binding/photoemulsion detection using [<sup>125</sup>I]-porcine galanin. The library consisted of 584 pools containing about 5,000 primary clones/pool for a total of about 3 million clones with an average insert size of 2.2 kb. Pools positive for rat GALR1 (about 110) were eliminated from the screen. Remaining pools were screened for radioligand binding using 1 nM [<sup>125</sup>I]-porcine galanin; slides were inspected for positive cells by direct microscopic examination. One positive pool (J126) was subdivided into 96 pools of about 90 clones each and rescreened for galanin binding. Preliminary pharmacology carried out on the positive subpool J126-10 indicated that the [<sup>125</sup>I]-porcine galanin binding was not sensitive to inhibition by galanin 3-29. 400 individual colonies of a positive pool (J26-10) were then screened to find two single purified cDNA clones. J126-10-334 was chosen for further analysis and designated K985. PCR analysis using three independent GALR1 primer sets (see Methods; data not shown) confirmed that the newly isolated cDNA

was distinct from GALR1 and thus encoded a new galanin receptor subtype, termed GALR2.

The isolated clone K985 carries a 3.8 kb insert.  
5 Sequence analysis of this cDNA revealed a complete coding region for a novel receptor protein which we term GALR2 (see Figures 1 and 2). Searches of GenEMBL databases indicated that the sequence was novel, and that the most similar sequence was that of the galanin receptor GALR1,  
10 followed by other G protein-coupled receptors (GPCR). The nucleotide and deduced amino acid sequences are shown in Figures 1 and 2, respectively. The nucleotide sequence of the coding region is ~56% identical to rat GALR1 and ~54% identical to human GALR1 and encodes a 372  
15 amino acid protein with 38% and 40% amino acid identity to rat and human GALR1, respectively. Hydropathy plots of the predicted amino acid sequence reveal seven hydrophobic regions that may represent transmembrane domains (TMs, data not shown), typical of the G protein-coupled receptor superfamily. In the putative TM  
20 domains, GALR2 exhibits 48-49% amino acid identity with rat and human GALR1. Like most GPCRs, the GALR2 receptor contains consensus sequences for N-linked glycosylation in the N-terminus (positions 2 and 11) as well as the  
25 predicted extracellular loop between TMs IV and V. The GALR2 receptor contains two highly conserved cysteine residues in the first two extracellular loops that are believed to form a disulfide bond stabilizing the functional protein structure (Probst et al., 1992).  
30 GALR2 shows five potential phosphorylation sites for protein kinase C in positions 138, 210, 227, 319, and 364; and two cAMP- and cGMP-dependent protein kinase phosphorylation sites in positions 232 and 316. It should be noted that six out of the seven potential  
35 phosphorylation sites are located in predicted intracellular domains, and therefore could play a role in regulating functional characteristics of the GALR2

rec ptor (Probst et al., 1992).

Within the GALR2 cDNA K985 (J126-10-334) isolated from  
th rat hypothalamus library, the coding r gion of GALR2  
5 is interrupted by an intron of ~1 kb (Figures 3A, 3B, and  
3C). A cDNA containing an intron may be produced by the  
action of reverse transcriptase on an incompletely  
spliced form of messenger RNA. The heterologous  
expression of the complete protein product is not  
10 necessarily impeded by the presence of the intron in the  
coding region, because the intron can typically be  
spliced out prior to translation by the host cell  
machinery. In the case of the GALR2 cDNA, the location  
of the intron combined with clear consensus sequences for  
15 5' and 3' splice junctions (Figures 3A and 3B) confirm  
that the intervening sequence represents an intron. As  
shown in Figure 3C, splicing of the intron at the  
indicated sites recreates an open reading frame within a  
highly conserved region of the GPCR family, at the end of  
20 TMIII (LDR/Y). It is of interest to note that several  
GPCRs have previously been reported to contain introns at  
this location, including the human dopamine D3, D4, and  
D5 receptors, the rat substance P receptor, and the human  
substance K receptor (Probst et al., 1992). In  
25 particular, the rat 5-HT<sub>1</sub> receptor (Shen et al., 1993)  
contains an intron in exactly the same location as is now  
reported for GALR2, within the AG/G codon for the highly  
conserved amino acid arginine at the end of TMIII (Figure  
3C).

30 To explore the possibility that incompletely or  
alternately spliced forms GALR2 mRNAs are present in the  
rat brain, RT-PCR using GALR2 PCR primers that are  
located in the coding region but that span the location  
35 of the intron was carried out. The sequences of the PCR  
primers are :

KS-1515 (Forward primer): 5'-CAAGGCTGTTTCATTTCTCATCTTTC  
(loop between TMs II and III) (SEQ.ID No. 12).

5 KS-1499 (Reverse primer): 5'-TTGGAGACCAGAGCGTAAACGATGG  
(end of TMVII) (SEQ.ID No. 13).

10 The PCR products were separated by gel electrophoresis,  
blotted, and hybridized with a radiolabeled  
oligonucleotide probe representing the predicted loop  
between TMs V and VI. The sequence of the  
oligonucleotide is:

15 KS-1540: 5'-AGTCGACCCGGTGACTGCAGGCTCAGGTTCCCAGCGCGCCAAACG  
(SEQ. ID No. 14).

RT-PCR analysis of GALR2 mRNA from various rat brain  
regions as described above indicates the existence of PCR  
products that may represent both the intronless (spliced)  
and intron-containing (incompletely spliced) forms of  
20 GALR2 (Figure 5). In addition, PCR products intermediate  
in size between intronless and intron-containing products  
that hybridize at high stringency with the GALR2  
oligonucleotide probe KS-1540 are present and may  
represent additional variations in the GALR2 mRNA. One  
25 mechanism that could generate such variations is  
alternative splicing. These results suggest that  
intronless transcripts exist in native tissue. A full-  
length intronless cDNA encoding the rat GALR2 receptor  
has been amplified and subcloned from rat heart RNA,  
30 which when transiently or stably transfected into cells  
binds galanin with high affinity.

#### Northern Blot Analysis of GALR2 mRNA

35 To define the size and distribution of the mRNA encoding  
GALR2 Northern blot analysis of poly A<sup>+</sup> RNA from various  
rat tissues and brain regions was carried out. A ~1.2 kb  
fragment of rat GALR2 containing the entire coding region



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but not containing the intron (Figur 1) was radiolabeled by random priming and used as a hybridization probe. Northern blots containing rat poly A<sup>+</sup> RNA were hybridized at high stringency and apposed to film. A single transcript of -1.8-2.0 kb is detected after a 4 day exposure of the autoradiogram at -80°C using Kodak Biomax MS film with one Biomax MS intensifying screen. Within the brain, the highest levels of GALR2 mRNA appear in hypothalamus (Figure 6A). Among various rat tissues, the GALR2 transcript is widely but unevenly distributed: GALR2 mRNA is observed in brain, lung, heart, spleen, and kidney, with lighter bands in skeletal muscle, liver, and testis (Figure 7A). Both Northern blots were reprobbed with 1B15 to confirm that similar amounts of mRNA were present in each lane (Figures 6B and 7B).

#### Pharmacological characterization of GALR2

The pharmacology of GALR2 was studied in COS-7 cells transiently transfected with the GALR2 cDNA, K985. Membrane preparations of Cos-7 cells transfected with K985 displayed specific binding to [<sup>125</sup>I]porcine galanin. Scatchard analysis of equilibrium saturation binding data yielded a K<sub>d</sub> = 150 pM with a B<sub>max</sub> = 250 fmol/mg protein. The pharmacological properties of the protein encoded by the GALR2 cDNA were probed by measuring the binding affinities of a series of galanin analogs, and compared to those of the rat GALR1 receptor expressed in the same host cell line. As shown in Table 1, both GALR1 and GALR2 receptors showed a high affinity for galanin<sub>(1-29)</sub>, the physiological ligand of these receptors. Both receptors also displayed high affinity for the truncated analogs galanin<sub>(1-16)</sub> and galanin<sub>(1-15)</sub>. Furthermore, the binding of [<sup>125</sup>I]porcine galanin to either GALR1 or GALR2 at concentrations up to 100 μM was not displaced by porcine galanin<sub>(3-29)</sub>. However, the GALR2 receptor has 540- and 4200-fold higher affinity for [D-Trp<sup>2</sup>]porcine galanin<sub>(1-29)</sub>, and [D-Trp<sup>2</sup>]galanin<sub>(1-16)</sub>, respectively, than

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the GALR1 subtype. Also, [Ala<sup>5</sup>]galanin<sub>(1-16)</sub>, and [Phe<sup>2</sup>]galanin<sub>(1-15)</sub> were moderately selective, with 15- and 17-fold greater affinities for the GALR2 receptor than for the GALR1 receptor subtype, respectively. [Ala<sup>9</sup>]galanin<sub>(1-16)</sub> was the only analog that was found to have the opposite selectivity, with 70-fold higher affinity for the GALR1 receptor than for the GALR2 receptor. Interestingly, these two receptor subtypes showed no significant differences in their binding affinities for the chimeric galanin antagonists, galantide, C7, M32, M35, and M40.

In LM(tk-) cells stably expressing the rat GALR2 receptor cDNA, porcine galanin<sub>(1-29)</sub> was found to inhibit the formation of cyclic AMP induced by 10  $\mu$ M forskolin. The effects of galanin were dose dependent with an  $EC_{50} = 0.26 \pm 0.13$  nM (n=3) (Figure 9A). In the same cell line porcine galanin<sub>(1-29)</sub> stimulated the formation of [<sup>3</sup>H]inositol phosphates, with an  $EC_{50} = 112$  nM (Figure 9B). The phosphoinositide response mediated by the rat GALR2 receptor suggests that this receptor can also couple to the intracellular calcium mobilization and diacylglycerol pathway. However, the 400-fold lower  $EC_{50}$  of porcine galanin<sub>(1-29)</sub> suggests that the GALR2 receptor couples with low efficiency to this signaling pathway. In support of this notion stands the observation that porcine galanin<sub>(1-29)</sub> had no effect on intracellular calcium levels in COS-7 cells transfected with the cDNA encoding the rat GALR2 receptor. Thus, the data presented herein suggest that the GALR2 receptor couples preferentially to  $G_{i\alpha}$ , since the stimulation of phosphoinositide metabolism and intracellular calcium mobilization are a hallmark of receptors to the  $G_q$  family of G-proteins. Furthermore, the data presented herein also indicate that the inhibition of cAMP formation, as well as the stimulation of phosphoinositide metabolism, can be used as functional assays to measure receptor activity in heterologous cell

systems expressing the rat GALR2 receptor.

In subsequent experiments, the inhibitory effect of rat GALR2 receptor stimulation on forskolin-stimulated cAMP accumulation in LM(tk-) cells could not be reproduced. However, the same LM(tk-) cells yielded a reproducible PI hydrolysis response (Table 4), and in independent binding assays a  $B_{max}$  of 4000 fmol/mg protein and a  $K_d$  of 1.1 nM when incubated with porcine  $^{125}$ I-galanin. It is concluded that in the cell lines studied thus far, the rat GalR2 is coupled primarily to the activation of phospholipase C and subsequent inositol phosphate metabolism, presumably through Gq or a related G protein. The PI response was evident as well in LM(tk-) cells stably transfected with the rat GALR2 receptor cDNA lacking an intron in the coding region (L-rGALR2I-4, see Table 4); membranes from these cells were shown in an independent experiment to bind porcine  $^{125}$ I-galanin with a  $B_{max}$  of 4800 fmol/mg membrane protein and a  $K_d$  of 0.2 nM.

The CHO cell line stably transfected with the rat GALR2 receptor (C-rGALR2-79) provided additional detail about the binding and signalling properties of the receptor. Membranes from stably transfected CHO cells were bound saturably by porcine  $^{125}$ I-galanin with a  $B_{max}$  of 520 fmol/mg membrane protein and a  $K_d$  of 0.53 nM. Peptides displaced the porcine  $^{125}$ I-galanin (Table 5) with binding affinities similar to those generated from transiently transfected COS-7 cells (Table 1). Receptor stimulation resulted in phosphatidyl inositol hydrolysis but had no effect on cAMP accumulation, again supporting the proposal that the rat GALR2 receptor is coupled primarily to phospholipase C activation through Gq or a related G protein. It was further demonstrated that rat GALR2 receptor activation could be monitored by arachidonic acid release (Table 5). Of interest, it was observed that the  $EC_{50}$  values from the PI hydrolysis assays were larger than the  $K_i$  values from

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binding assays whereas the  $EC_{50}$  values from the arachidonic acid assays were comparable to the binding data. One possibility suggested by this data is that the calcium release induced by inositol phosphate metabolism leads to activation of phospholipase A2 and subsequently to the hydrolysis of arachidonic acid from membrane phospholipids. The lower  $EC_{50}$  values in the arachidonic acid assays may reflect an amplification process in the second messenger pathway, such that a maximal arachidonic acid response occurs at submaximal calcium concentrations.

The stably transfected CHO cells were used to further explore the binding and signalling properties of the rat GalR2 receptors (Table 6). The peptide binding profile was similar to that generated previously with transiently transfected COS-7 cells. Porcine, rat and human galanin bound with high affinity as did C-terminally truncated peptides as short as galanin 1-12. Chimeric or putative "antagonist" peptides including C7, galantide, M32, M35 and M40 displayed relatively high binding affinity except for C7 ( $K_i = 47$  nM). Galanin analogs containing D-Trp<sup>2</sup> (D-Trp<sup>2</sup>-galanin 1-29 and D-Trp<sup>2</sup>-galanin 1-16) retained measurable binding affinity ( $K_i = 41$  and 110 nM, respectively). The N-terminally truncated peptide galanin 3-29 was inactive.

Selected peptides were subsequently tested in the arachidonic acid release assays. Peptides with measurable  $EC_{50}$  values mimicked the maximal effect of rat galanin (1  $\mu$ M) on arachidonic acid release and were classified as full agonists, including C7, galantide, M32, M35 and M40. The binding and functional profiles were in general agreement. Notable exceptions include D-Trp<sup>2</sup>-galanin 1-29, D-Trp<sup>2</sup>-galanin 1-16, and C7, all of which gave larger  $K_i$  values vs.  $EC_{50}$  values; one possibility is that these peptides were less stable in

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the binding assay vs. the functional assay. It is, therefore, concluded that the arachidonic acid release assay is useful for assessing peptide potency and intrinsic activity for the rat GalR2 receptor when stably expressed in CHO cells.

Peptides were further evaluated for their ability to selectively activate the rat GALR1 receptor (monitored in stably transfected LM(tk-) cells using the forskolin-stimulated cAMP accumulation assay) vs. the rat GALR2 (monitored in stably transfected CHO cells using the arachidonic acid release assay). Data are reported in Table 7. D-Trp<sup>2</sup>-galanin was 8.5-fold less potent than galanin in the rat GALR2 functional assay but > 15000-fold less potent than galanin in the rat GALR1 functional assay. Similarly, D-Trp<sup>2</sup>-galanin 1-16 was 38-fold less potent than galanin in the rat GALR2 functional assay but > 170,000-fold less potent than galanin in the rat GALR1 functional assay. It is concluded that D-Trp<sup>2</sup>-galanin and analogous peptides may serve as useful tools with which to explore the function of GALR2 vs. GALR1 receptors in native tissues and physiological systems.

#### Feeding Assays

Rats were injected icv with either galanin, galanin derivatives, or saline. Cumulative food intake was measured over a period of 3 hours. Baseline food intake associated with the saline control was 1.5 gram. A maximal food intake of 6.81 grams was observed after a 10 nmole injection of galanin. The ED50 for galanin is estimated to be 1 nmole. M40 was also tested in this paradigm. M40 was able to mimic the effects of galanin, with a maximal food intake of 6.3 grams observed after a 50 nmol injection. The ED50 for M40 is estimated to be 20 nmoles.

#### Heterologous expression of GPCRs in Xenopus oocytes

Heterologous expression of GPCRs in Xenopus oocytes has been widely used to determine the identity of signaling pathways activated by agonist stimulation (Gundersen et al., 1983; Takahashi et al., 1987). Application of porcine galanin (100-1000 nM) activates rapid inward currents in 36 of 46 oocytes injected with 5-50 pg rGalR2 mRNA (Fig. 13). Equimolar concentrations of C7 induces similar currents whereas galanin 3-29 is inactive (0/11 oocytes). Oocytes injected with buffer (ND96) alone or 5-HT<sub>1a</sub> receptor mRNA do not exhibit detectable (< 5 nA) responses to galanin (0/19). Current magnitudes in rGalR2 mRNA-injected oocytes range from small fluctuations of less than 50 nA (excluded from analysis) to large rapid currents (up to 3  $\mu$ A) resembling those activated by stimulation of other receptors (alpha receptors - data not shown) that are known to couple to IP<sub>3</sub> release and stimulation of Cl<sup>-</sup> current from the resulting increase in intracellular free Ca<sup>2+</sup> (Takahashi et al., 1987). The currents stimulated by galanin in oocytes expressing rGalR2 are most likely mediated by the endogenous calcium-activated Cl<sup>-</sup> channel (Gundersen et al., 1983) because they are blocked in oocytes injected with 50 nl of 10 mM EGTA (5/5) and they display a current-voltage relation that exhibits outward rectification and a reversal potential of approximately -15 mV (data not shown).

#### Receptor autoradiography

The relative proportion of the total [<sup>125</sup>I]galanin binding attributable to the GALR2 receptor was determined as the binding which was removed by 60 nM [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub>. The numerical representations in Table 2 indicate: 1) the relative intensity of the total binding obtained with [<sup>125</sup>I]galanin, with +3 being the maximum; and 2) the relative amount of this binding attributable to GALR2, with +3 again being the maximum.

Total [ $^{125}$ I]galanin binding was observed in many regions of the rat brain, and was especially intense in the forebrain, including the amygdala, parts of the hypothalamus and thalamus, the septum, and the ventral hippocampus. Other regions with intense binding signals included the superior colliculus, the central gray, and the dorsal horn of the spinal cord. The inclusion of 5  $\mu$ M porcine galanin in the incubation resulted in a complete displacement of [ $^{125}$ I]galanin binding from the rat brain tissue sections. The use of 60 nM [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> partially displaced [ $^{125}$ I]galanin binding from many regions of the rat brain.

The areas most affected by the GALR2 selective ligand were the lateral septum, the paraventricular hypothalamic nucleus, the centromedial and centrolateral thalamic nuclei, the amygdalopiriform area of the amygdala, and the superior colliculus. Other forebrain regions with lesser but still significant reductions in [ $^{125}$ I]galanin binding included the piriform and entorhinal cortices, the globus pallidus, the supraoptic, lateral, and ventromedial hypothalamic nuclei, and the anterior, cortical, medial, and central amygdaloid nuclei. In the midbrain, pons and medulla, [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> partially reduced the total binding in the central gray, the raphe obscurus and raphe magnus, the parabrachial nucleus, the pontine reticular formation, the hypoglossal nucleus, and the gigantocellular reticular nucleus.

In contrast, there were a number of areas in which [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> had little or no effect on the total [ $^{125}$ I]galanin binding. Of these, the most striking were the nucleus of the lateral olfactory tract, the ventral hippocampus, and the dorsal horn of the spinal cord. Other areas in which significant binding remained included the olfactory bulb, the insular cortex, the islands of Calleja, the nucleus accumbens, the lateral

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habenula, the arcuate nucleus, and the spinal trigeminal nucleus.

#### Developmental in situ hybridization

5 Using oligonucleotide probes, GalR2 mRNA appeared to be developmentally regulated. At P1 and P5, film autoradiography of the hybridized brain sections revealed clear signals over many thalamic nuclei. In the hypothalamus, both the paraventricular and ventromedial  
10 nuclei were labeled. In addition, the superficial layers of neocortex contained visible hybridization signal, as did the dorsal hippocampus. In the mesencephalon, a low level of hybridization signal was observed in the pretectal region.

15

#### Ribonuclease protection assay

RNA was isolated and assayed as described from: heart, striated muscle, liver, kidney, and CNS regions. CNS regions included: spinal cord, amygdala, hypothalamus, cerebral cortex, cerebellum, and hippocampus. The  
20 highest levels of rGalR2 were detected in the hypothalamus (Figure 14). Lower amounts were found in heart, kidney, hippocampus amygdala, spinal cord, and cerebellum (Figure 14). mRNA coding for the rGalR2 was  
25 not detected in RNA extracted from striated muscle or liver.

#### Generation of Human GALR2 PCR product:

Using PCR primers designed against the fourth and sixth transmembrane domains of the rat GALR2 sequence, NS 525  
30 and NS526, a 300 base pair fragment was amplified from 3 different lots of human genomic DNA. Sequence from all three human genomic DNAs were >98% identical and displayed 84% nucleotide identity to the rat GALR2 gene,  
35 between the second extracellular domain and the 5' end of the sixth transmembrane. This level of homology is typical of a species homologue relationship in the GPCR



sup rfamily.

#### 5' and 3' RACE Analysis of Human GALR2

5' RACE was performed on human brain RNA to isolate hGALR2 sequence upstream of the genomic PCR product above. Using nested reverse primers from the fifth transmembrane domain of hGALR2, a 600 base pair fragment was amplified. The sequence of this RACE product displayed 91% nucleotide identity to rGALR2 from the 3' end of the second transmembrane domain to the 5' end of the fifth transmembrane domain.

3' RACE was performed on human lung RNA to determine the sequence of the COOH terminus of hGALR2. Using nested forward primers from the fifth transmembrane domain of hGALR2, a 500 bp RACE product was generated that showed a 77% identity to nucleotides 1080-1139 of rGALR2. The sequence of this RACE product downstream from this region showed less homology to rGALR2, and was presumed to represent the COOH terminus and 3' UT of the hGALR2 gene.

#### Construction and Screening of a Human Heart cDNA Library

To obtain a full-length hGALR2 clone, superpools of a human heart cDNA library were screened by PCR using primers BB153 and BB169. A 325 base pair fragment was amplified from superpools 6, 9 and 16. Two positive primary pools, 69 and 72, were identified from superpool 9, and 1 positive primary pool, 121, was identified from superpool 16. One positive primary pool, 69, was subdivided into 48 pools of 3333 individual clones and screened by PCR. Twelve positive subpools were identified and one, 69-11, was subdivided into 20 pools of 1200 clones, plated onto agar plates, and screened by southern analysis. Thirty colonies that appeared positive were rescreened by PCR using primers BB167 and BB170, revealing 4 positive colonies. One of these, 69-11-5 was chosen for further analysis. To evaluate

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whether this colony represented a single clone, a dilution of the colony was amplified on agar plates and colonies were screened by PCR using primers BB167 and BB170. Five of 20 colonies were positive for hGALR2, indicating that 69-11-5 was a mixture of 2 or more clones. One positive colony, 69-11-5-3, designated B029, was amplified as a single hGALR2 clone. Vector-anchored PCR revealed that B029 is in the correct orientation for expression, and encodes approximately 200 base pairs of 5'UT and 5000 base pairs 3'UT. Preliminary single-stranded sequence analysis indicates that B029 encodes an initiating methionine and a termination codon, and contains an intron between the third and fourth transmembrane domains which is approximately 1.2kb in length. 69-11-5 has been demonstrated to confer  $^{125}\text{I}$  galanin binding in transfected COS-7 cells, as assessed by microscopic analysis of photoemulsion-dipped slides. In addition, COS-7 cells transfected with the single clone B029 exhibit significant binding of  $^{125}\text{I}$  galanin in comparison with COS-7 cells transfected with control vector. In preliminary radioligand binding experiments,  $^{125}\text{I}$  porcine galanin bound to membranes from COS-7 cells transfected with B029, with a specific binding of 4900 fmol/mg, when the membranes (0.005 mg/ml) were incubated with 0.4 nM porcine galanin for 30 min. at 30° C. No specific binding was detected to membranes from mock-transfected COS-7 cells when tested under the same conditions.

### 30 Human GALR2 receptor pharmacology

A human GALR2 receptor construct containing an intron in the coding region of the cDNA (B029) was prepared and transiently transfected into COS-7 cells. Human GALR2 receptors expressed in the COS-7 cell membranes were labeled by porcine  $^{125}\text{I}$ -galanin with an apparent  $B_{\text{max}}$  of 4200 fmol/mg membrane protein and a  $K_d$  of 0.97 nM. The peptide binding profile for the human GALR2 receptor

(Table 8) resembled that reported previously for the rat GALR2 in COS-7 cell membranes (Table 1).

A human GALR2 receptor cDNA construct lacking the intron in the coding region was also prepared (BO39) and transiently transfected into COS-7 cells. In a preliminary experiment, membranes from transiently transfected cells (membrane protein concentration = 0.045 mg/ml) were incubated with porcine  $^{125}\text{I}$ -galanin (0.17 nM), and specific binding was measured as 480 fmol/mg membrane protein. Assuming an estimated  $K_d$  of 1 nM, the estimated  $B_{\text{max}}$  for this construct would be ~3400 fmol/mg membrane protein. Therefore, it is concluded that the absence of the intron in the coding region of the human GALR2 cDNA has no significant effect on receptor expression or porcine  $^{125}\text{I}$ -galanin binding.

#### Experimental Discussion

In order to clone additional members of the galanin receptor family, an expression cloning strategy based on the potential presence of multiple galanin receptors in the hypothalamus was designed. Using this strategy a cDNA clone encoding a galanin receptor from rat hypothalamus, termed GALR2, was isolated that is distinct from the previously cloned GALR1 receptors.

Transient transfection of the isolated cDNA (K985) encoding GALR2 resulted in high affinity binding of [ $^{125}\text{I}$ ]-porcine galanin. The high binding affinity of the GALR2 receptor for galanin<sub>(1-29)</sub> and its truncated analogs galanin<sub>(1-16)</sub> and galanin<sub>(1-15)</sub> strongly supports the notion that the GALR2 receptor is a novel galanin receptor subtype. Both the rat GALR1 and GALR2 receptors seem to bind preferentially to the amino terminus of galanin. Deletion of 13 or 14 amino acids from the carboxyl terminus of galanin still yields peptides with high binding affinity at both the GALR1 and GALR2 receptors.

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Furthermore, the truncation of the first two amino acids of the amino terminus led to a complete loss of affinity at both GALR1 and GALR2. Consistent with this notion are the findings that the chimeric peptides, which share identical amino acid sequences in the first 12 amino acids with galanin had very similar binding affinities for either GALR1 or GALR2 receptors. In spite of these similarities, the substitution of L-tryptophan with D-tryptophan in position 2 of porcine galanin<sub>(1-29)</sub> ([D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub>) led to a 7,000-fold loss in affinity at the GALR1 receptor compared to only a 14-fold reduction at the GALR2 receptor. The same substitution in the truncated analog galanin<sub>(1-16)</sub> led to a 4,200-fold reduction in affinity at the GALR1 receptor, and only a 6-fold reduction in affinity at the GALR2 receptor. These data suggest that galanin analogs, with modifications at the 2-position, are better tolerated at the GALR2 receptor than at the GALR1 receptor as long as the side chain is an aromatic moiety.

Conversely, the substitution of tyrosine with alanine in position 9 of galanin<sub>(1-16)</sub>, (i.e., to make [Ala]<sup>9</sup> galanin) leads to a 680-fold reduction in affinity at the GALR1 receptor and to a 60,000-fold reduction in affinity at the GALR2 receptor. Altogether, the major differences in binding selectivity of the substituted analogs of galanin suggest the existence of substantial differences in the binding domains of these two receptor subtypes.

The existence of such structural differences between the GALR1 and GALR2 receptors are indicative of the potential for the design and discovery of novel subtype selective compounds. In this regard, the expression of the cDNA encoding the rat GALR2 receptors in cultured cell lines provides a unique tool for the discovery of therapeutic agents targeted at galanin receptors.

Localization of galanin receptors

The high affinity of [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> for the cloned GALR2 receptor (6 nM), and its low affinity for the GALR1 receptor (3  $\mu$ M), makes it a useful tool for receptor autoradiographic studies. Thus, brain areas in which the total [<sup>125</sup>I]galanin binding is significantly reduced by [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub> are interpreted as areas containing a high proportion of GALR2 receptors, or other galanin receptors with similar high affinity for [D-Trp<sup>2</sup>]galanin<sub>(1-29)</sub>. Those with lesser reductions are seen as regions containing a higher concentration of GALR1 receptors. The lateral septum, the paraventricular hypothalamic nucleus, the centromedial and centrolateral thalamic nuclei, the amygdalopiriform area of the amygdala, and the superior colliculus all appear to contain primarily GALR2 receptors. In contrast, the nucleus of the lateral olfactory tract, the ventral hippocampus, and the dorsal horn of the spinal cord appear to contain primarily GALR1 receptors. The predominance of the GALR1 receptor in these regions is consistent with published reports of the GALR1 messenger RNA localization (Parker et al., 1995; Gustafson et al., 1996). In most other regions, there appears to be a significant overlap between the two subtypes.

While the functional implications of the GALR2 receptor localization are not well understood at present, there are a number of physiological processes attributable to galanin that could be mediated by this receptor. These include feeding (paraventricular hypothalamic nucleus), cognition (septum and hippocampus), analgesia and/or sensory processing (midline thalamic nuclei), and anxiety and depression (amygdala and hypothalamus).

The observation that galanin is co-released with norepinephrine from sympathetic nerve terminals suggests that galanin could act via galanin receptors in the

periphery to modulate nearly every physiological process controlled by sympathetic innervation. Additional therapeutic indications not directly related to localization (supra) include diabetes, hypertension, cardiovascular disorders, regulation of growth hormone release, regulation of fertility, gastric ulcers, gastrointestinal motility/transit/absorption/secretion, glaucoma, inflammation, immune disorders, respiratory disorders (eg. asthma, emphysema).

The physiological and anatomical distribution of galanin-containing neurons suggests potential roles of galanin receptors mediating effects on cognition, analgesia, neuroendocrine regulation, control of insulin release and control of feeding behavior. Of particular relevance to the role of the novel GALR2 receptor, are those functions mediated by galanin receptors in the rat hypothalamus.

Studies in rats indicate that the injection of galanin in the hypothalamus increases food intake (Kyrouli et al, 1990, and Schick et al, 1993) and that this stimulatory effect of galanin is blocked by prior administration of M40 and C7 (Liebowitz and Kim, 1992; and Corwin, 1993). The expression of the mRNA encoding the GALR1 receptor in the rat hypothalamus, (Parker et al., 1995, Gustafson et al., 1996) and the fact that the novel GALR2 receptor was cloned from a cDNA library prepared from rat hypothalamus argues in favor of either receptor subtype to be involved in the regulation of feeding behavior (Parker et al., 1996). However, the evidence against the involvement of GALR1 in the stimulation of feeding behavior stems from the fact that M40 and C7 are known to be agonists, and not antagonists, in cell lines expressing the cloned human and rat GALR1 receptors (Heuillet et al. 1994; Hale et al. 1993; and Bartfai et al. 1993).

The distribution of GALR2 mRNA in the rat brain and

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p riph ry has been d termed by ribonuclease protection assay, in situ hybridization, Northern blot analysis and RT-PCR. The results of these studies suggest that this recept r is potentially involved in mediating many of the physiological roles ascribed to the peptide galanin. In the adult rat, localization of the GalR2 mRNA in the hypothalamus indicates a role for this receptor in homeostatic mechanisms, including food intake and neuroendocrine regulation. The presence of GALR2 mRNA in the neocortex and dorsal hippocampus suggest an involvement in cognition, which is consistent with documented changes in galanin and galanin receptor expression during aging and in the brains of Alzheimer's patients (Chan-Palay, 1988; Leverenz et al., 1996). Galanin also has antinociceptive effects, and the localization of GALR2 mRNA in the spinal cord (present investigation) and dorsal root ganglia (O'Donnell et al., 1996) implicate this receptor in pain neurotransmission. The localization of GALR2 mRNA in the cerebellum is intriguing, as it suggests a role for galanin and the GalR2 receptor in planned movements and potentially in movement disorders.

In addition to the localization observed in adult animals, it also appears that the GALR2 mRNA is developmentally regulated, with the highest levels observed early in postnatal development. Thus, it is possible that this galanin receptor plays a role in developmental processes which occur during the first postnatal week, such as axonal guidance and synapse formation.

A unique pharmacological profile for the GALR2 receptor has been generated through binding and functional assays. This profile can be used to deduce the physiological function of the GalR2 receptor in vivo. Consider the agonist activity of galanin 1-16, for example. Galanin

1-16 is reported to function as an agonist in various models of hypothalamic, pituitary and pancreatic function (Kask et al.) Galanin 1-16 is also reported to mimic the effects of galanin on the flexor reflex in the rat.

5 The N-terminally extended peptides galanin (-7) to (+) 29 and galanin (-9) to (+) 29, also characterized as rat GALR2 agonists, can mimic the effects of galanin in a rat flexor reflex assay (Weisenfeld). Taken together, these data suggest a potential role for the rat GalR2 receptor in a range of physiology or pathophysiology including diabetes, pain, reproduction, obesity and eating disorders.

15 The agonist activity of M40 in GALR2 in vitro assays is particularly intriguing when viewed in the context of behavioral feeding models. From the literature, one might conclude that the agonist activity of M40 in vitro is in apparent conflict with the antagonist activity reported for M40 in behavioral models of food intake, and that the GALR2 receptor is therefore unlikely to mediate the feeding response. The data generated and reported in the subject application do not support this conclusion. Rather, the data from behavioral feeding models indicate that M40 is an orexigenic peptide whose maximal effect is comparable to that for galanin itself. The agonist activity reported herein for M40 both in vitro and in vivo is consistent with the proposal that the rat GALR2 receptor mediates the stimulatory effect of galanin on food intake in the central nervous system. These data further suggest that the rat GALR2 receptor represents a target for the design of therapeutic compounds for the treatment of obesity and related disorders.



Table 1: Binding of galanin peptide analogs to the recombinant rat GALR1 and GALR2 receptors transiently expressed in COS 7 cells.

Analog	GALR1 (pKi)		GALR2 (pKi)	
	Mean	SEM*	Mean	SEM
porcine galanin (1-29)	9.34	0.15	9.35	0.14
[D-Trp <sup>2</sup> ]porcine galanin (1-29)	5.46	0.04	8.19	0.26
[Phe <sup>2</sup> ]porcine galanin (1-29)	5.99	0.13	5.64	0.11
[D-Ala <sup>7</sup> ]porcine galanin (1-29)	8.66	0.04	8.76	0.09
galanin (1-16)	8.66	0.01	8.76	0.13
[D-Trp <sup>2</sup> ]galanin (1-16)	4.40	0.09	8.02	0.10
[Ala <sup>5</sup> ]galanin (1-16)	6.27	0.05	7.46	0.13
[Ala <sup>9</sup> ]galanin (1-16)	5.83	0.02	3.98	0.10
galanin (1-15)	8.47	0.04	9.19	0.06
[Phe <sup>2</sup> ]galanin (1-15)	4.63	0.03	5.85	0.49
porcine galanin (3-29)	< 4.0		< 4.0	
galantide	8.02	0.08	8.70	0.07
C-7	7.79	0.01	7.72	0.09
M32	9.21	0.10	9.23	0.05
M35	9.48	0.07	9.24	0.10
M40	8.44	0.09	9.14	0.21

\*SEM=standard error of the mean, from 3 independent experiments.

Tabl 2. Distribution of [ $^{125}$ I]galanin binding in rat brain. Total binding is compared to the amount attributable to GALR2 (as indicated by displacement of [ $^{125}$ I]galanin by 60 nM [D-Trp<sup>2</sup>]porcine galanin<sub>(1-29)</sub>).

Region	Total [ $^{125}$ I]Gal binding	Putative GALR2 sites	Potential Applications
Olfactory bulb	+3	+1	Modulation of olfactory sensation
Anterior olfactory n.	+3	+1	Modulation of olfactory sensation
Cortex			
dorsal neocortex, layer 4	+1	+1	Sensory integration
piriform	+2	+1	Modulation of olfactory sensation
agranular insular	+3	+1	Processing of visceral information
entorhinal	+2	+1	
dorsal endopiriform	+2	+1	
Claustrum	+2	+1	Visual processing
Basal ganglia			
n. accumbens	+2	0	Modulation of dopaminergic function
olfactory tubercle	+2	+1	
globus pallidus	+1	+1	
islands of Calleja	+3	+1	
Septal area			
lateral septum	+3	+2	Cognitive enhancement via cholinergic system
diagonal band n.	+2	0	
Hypothalamus			
anterior	+1	0	Neuroendocrine regulation
supraoptic n.	+2	+1	
paraventricular	+2	+2	Appetite/obesity
ventromedial	+2	+1	

R gi n	T tal [ <sup>125</sup> I]Gal binding	Putativ GALR2 sites	P tential Applicati ns
arcuate	+1	0	
lateral	+2	+1	
medial mammillary	+2	+1	
Thalamus			Analgesia/sensory modulation
paraventricular n.	+1	0	
centromedial	+3	+2	
paracentral	+3	+1	
rhomboid	+1	0	
reuniens	+2	+1	
mediodorsal	+2	0	
reticular n.	+1	+ $\frac{1}{2}$	
centrolateral n.	+3	+2	
zona incerta	+2	+1	
lateral dorsal	+1	+ $\frac{1}{2}$	
habenula	+3	+1	Anxiety/sleep disorders
Hippocampus			Cognition enhancement/ ischaemia
Cal, ventral	+3	0	
subiculum	+2	+1	
Amygdala			Anxiolytic, appetite, depression
bed n. stria terminalis	+3	+1	
n. lateral olfactory tract	+3	0	
Amygdala			Anxiolytic, appetite, depression
anterior	+2	+1	
medial	+3	+1	
cortical	+2	+1	
central	+3	+1	
amygdalohippocampal	+2	0	
amygdalopiriform	+3	+2	
Midbrain			

Region	Total [ <sup>125</sup> I]Gal binding	Putative GALR2 sites	Potential Applications
superior colliculus	+3	+2	Visual function
raphe obscurus	+2	+1	Analgesia
central gray	+2	+1	Analgesia
Pons/medulla			
raphe magnus	+2	+1	Analgesia
parabrachial n.	+2	+1	
pontine ret. n.	+2	+1	
reticulotegmental	+2	+1	
gigantocellular	+2	+1	
motor trigeminal	+1	0	
spinal trigeminal	+3	+1	Migraine
hypoglossal n.	+2	+1	Motor coordination
area postrema	+1	0	
Spinal cord			
dorsal horn	+3	+1	Analgesia

Table 3. Northern blot hybridization of GALR2 receptor in brain and various peripheral rat tissues.

Tissue	Blot 1	Blot 2	Mean Signal	Therapeutic Indications
Heart	+++	++	2.5	Cardiovascular Indications (including hypertension and heart failure)
Brain	++++	++++	4.0	Obesity/feeding, analgesia, cognition enhancement, Alzheimer's disease, depression, anxiety, sleep disorders, Parkinson's disease, traumatic brain injury, convulsion/epilepsy
Spleen	++	++	2.0	Immune functions, hematopoiesis
Lung	++++	++++	4.0	Respiratory disorders, asthma, emphysema, lung cancer diagnostics
Liver	++	-	1.0	Diabetes
Skeletal Muscle	+	++	1.5	Diabetes
Kidney	+++	+++	3.0	Hypertension, electrolyte balance, diuretic, anti-diuretic
Testis	+++	+	2.0	Reproductive function

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Table 4. Inositol phosphat hydrolysis in LM(tk-) cells stably transfected with GALR2.

P ptide	EC <sub>50</sub> PI (nM) rat GALR2 (with intron)	EC <sub>50</sub> PI (nM) L-rGALR2I-4 (intronless)
porcine galanin	21	14
M35	29	28
D-Trp <sup>2</sup> -galanin 1-16	1380	660
D-Trp <sup>2</sup> -galanin 1-29	200	230
galanin 1-16	65	18
M40	28	47
M32	13	35

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Table 5. Rat GALR2 receptors stably transfected in CHO  
Comparison of binding data, phosphatidyl inositol  
release, and arachidonic acid release in C-rGalR2-79.

Peptide	K <sub>i</sub> from porcine <sup>125</sup> I-galanin binding assays (nM)	EC <sub>50</sub> from PI hydrolysis assays (nM)	EC <sub>50</sub> from arachi- donic acid assays (nM)
rat galanin	0.52	14	0.67
porcine galanin	0.94	15	1.3
porcine galanin 1-16	3.5	91	2.6
D-Trp <sup>2</sup> -galanin 1-16	110	590	50

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Table 6. CHO GALR2 pharmacology: binding ( $K_i$  vs.  $^{125}$ I-porcine galanin) vs. function (arachidonic acid hydrolysis)

Peptide	Rat GALR2 $K_i$ , C-rGalR2-79 (nM)	Rat GALR2 EC <sub>50</sub> AA, C-rGalR2-79 (nM)
Human galanin	1.2	
Porcine galanin	0.94	1.3
rat galanin	0.52	0.67
porcine gal -7 to + 29		3.0
porcine galanin -9 to + 29		4.0
porcine galanin 3-iodo-L-Tyr9-galanin		0.8
porcine galanin 3-iodo-L-Tyr26 galanin		1.0
porcine Phe2-galanin	> 1000	
porcine D-Trp2-galanin	41	11
D-Trp2-3-iodo-L-Tyr9-galanin		3.0
porcine D-Trp2-3-iodo-L-Tyr26-galanin		6.0
D-Ala7-galanin	6.2	5.4
porcine galanin 3-29	> 1000	> 1000
porcine galanin 9-29	> 1000	> 1000
porcine galanin 17-29	> 1000	
porcine galanin 1-16	3.5	2.6
porcine Ala2-galanin 1-16	> 1000	
porcine D-Trp2-galanin 1-16	110	50
porcine Ala5-galanin 1-16	> 620	
porcine Ala9-galanin 1-16	> 1000	
porcine galanin 1-15	1.5	2.3
Phe2-galanin 1-16	> 1000	> 1000
porcine galanin 1-12	2.1	2.3
porcin galanin 1-9	> 1000	> 1000



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Peptide	Rat GALR2 K <sub>i</sub> , C-rGalR2- 79 (nM)	Rat GALR2 EC <sub>50</sub> AA, C-rGalR2-79 (nM)
C7	48	2.4
Galantide	4.9	0.93
M32	3.4	2.5
M35	5.8	1.3
M40	3.5	2.7

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Table 7. Peptide-dependent activation of rat GALR1 vs. rat GALR2.

Peptid	LM(tk-) Rat GALR1 cAMP assay EC <sub>50</sub> (nM)	C-RGalR2-79 arachidonic acid assay EC <sub>50</sub> (nM)
Porcine galanin	0.06	1.3
rat galanin	0.05	0.67
porcine D-Trp <sup>2</sup> -galanin	> 850	11
porcine galanin 3-29	> 1000	> 1000
porcine galanin 1-16	0.34	2.6
porcine D-Trp <sup>2</sup> -galanin 1-16	> 1000	50
C7	0.52	2.4
Galantide	0.08	0.93
M32	0.34	2.5
M35	0.15	1.3
M40	0.82	2.7

Table 8. Peptide binding profile: Human GALR2 vs. rat GALR2 transiently expressed in COS-7

Peptide	Human GALR2 K <sub>i</sub> (nM)	Rat GALR2 K <sub>i</sub> (nM)
porcine galanin 1-16	15	7.2*
porcine galanin	0.72	0.45
M40	5.3	0.72
porcine D-Trp <sup>2</sup> -galanin	290	52*
M32	7.9	12*
rat galanin	1.0	0.52*

\*additional experiments were performed with some of the peptides shown in Table 1.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT: Smith, Kelli E.

Gerald, Christophe P.G.

Weinshank, Richard L.

Linemeyer, David

Branchek, Theresa

Forray, Carlos

(ii) TITLE OF INVENTION: DNA ENCODING GALANIN GALR2 RECEPTORS  
AND USES THEREOF

## (iii) NUMBER OF SEQUENCES: 32

## (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Cooper &amp; Dunham LLP

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(C) CITY: New York

(D) STATE: New York

(E) COUNTRY: U.S.A.

(F) ZIP: 10036

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: White, John P.

(B) REGISTRATION NUMBER: 28,678

(C) REFERENCE/DOCKET NUMBER: 50233-D-PCT/JPW/MAT

## (ix) TELECOMMUNICATION INFORMATION:

- 135 -

(A) TELEPHONE: 212-278-0400

(B) TELEFAX: 212-391-0525

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGGGCAACAG CCTAGTGATC ACCG

24

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTGCTCCCAG CAGAAGGTCT GGTT

24

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTCAGTGAA GGAATGGGA GCGA

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTCATTGCAA ACACGGCACT TGAACA

26

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTTGCTTGTA CGCCTTCCGG AAGT

24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAGAACTTCA TCACGCTGGT GGTG

24

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1119 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1119

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG AAT GGC TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC 48  
Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly

1

5

10

15

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GGT AGC GGC GGC TGG CAG CCT GAG GCG GTC CTT GTA CCC CTA TTT TTC 96  
 Gly Ser Gly Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe  
                   20                                  25                                  30

GCG CTC ATC TTC CTC GTG GGC ACC GTG GGC AAC GCG CTG GTG CTG GCG 144  
 Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala  
                   35                                  40                                  45

GTG CTG CTG CGC GGC GGC CAG GCG GTC AGC ACC ACC AAC CTG TTC ATC 192  
 Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile  
                   50                                  55                                  60

CTC AAC CTG GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCT 240  
 Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro  
                   65                                  70                                  75                                  80

TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG 288  
 Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu  
                                   85                                  90                                  95

CTC TGC AAG GCT GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC 336  
 Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser  
                   100                                  105                                  110

AGC TTC ACG CTG GCC GCC GTC TCC CTG GAC AGG TAT CTG GCC ATC CGC 384  
 Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg  
                   115                                  120                                  125

TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA AAC GCG CTG GCC 432  
 Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala  
                   130                                  135                                  140

GCC ATC GGG CTC ATC TGG GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC 480  
 Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr  
                   145                                  150                                  155                                  160

CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC 528  
 Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His  
                                   165                                  170                                  175

CCA GCA TGG AGC GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC 576  
 Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe



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180

185

190

GTC TTT AGC TAC CTG CTG CCA GTG CTA GTC CTC AGT CTG ACC TAT GCG 624  
Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Ser Leu Thr Tyr Ala  
195 200 205

CGT ACC CTG CGC TAC CTC TGG CGC ACA GTC GAC CCG GTG ACT GCA GGC 672  
Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro Val Thr Ala Gly  
210 215 220

TCA GGT TCC CAG CGC GCC AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC 720  
Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile  
225 230 235 240

GTG GCG GTG CTT TTC TGC CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC 768  
Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile  
245 250 255

CTC TGC GTG TGG TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG 816  
Leu Cys Val Trp Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala  
260 265 270

TTG CGC ATC CTT TCA CAC CTA GTT TCC TAT GCC AAC TCC TGT GTC AAC 864  
Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn  
275 280 285

CCC ATC GTT TAC GCT CTG GTC TCC AAG CAT TTC CGT AAA GGT TTC CGC 912  
Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg  
290 295 300

AAA ATC TGC GCG GGC CTG CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC 960  
Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly  
305 310 315 320

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CGA GTG AGC ATC CTG GCG CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA 1008  
 Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu  
 325 330 335

CAG GAA TCC ACA GAC CTG ACA CAG GTG AGC GAG GCA GCC GGG CCC CTT 1056  
 Gln Glu Ser Thr Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu  
 340 345 350

GTC CCA CCA CCC GCA CTT CCC AAC TGC ACA GCC TCG AGT AGA ACC CTG 1104  
 Val Pro Pro Pro Ala Leu Pro Asn Cys Thr Ala Ser Ser Arg Thr Leu  
 355 360 365

GAT CCG GCT TGT TAA 1119  
 Asp Pro Ala Cys \*  
 370

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 373 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly  
 1 5 10 15

Gly Ser Gly Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe  
 20 25 30

Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala  
 35 40 45

Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile  
 50 55 60

Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro  
 65 70 75 80

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Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu  
85 90 95

Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser  
100 105 110

Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg  
115 120 125

Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala  
130 135 140

Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr  
145 150 155 160

Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His  
165 170 175

Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe  
180 185 190

Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Ser Leu Thr Tyr Ala  
195 200 205

Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro Val Thr Ala Gly  
210 215 220

Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile  
225 230 235 240

Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile  
245 250 255

Leu Cys Val Trp Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala  
260 265 270

Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn  
275 280 285

Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg  
290 295 300

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Lys Ile Cys Ala Gly Leu L u Arg Pro Ala Pro Arg Arg Ala Ser Gly  
 305 310 315 320

Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu  
 325 330 335

Gln Glu Ser Thr Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu  
 340 345 350

Val Pro Pro Pro Ala Leu Pro Asn Cys Thr Ala Ser Ser Arg Thr Leu  
 355 360 365

Asp Pro Ala Cys \*

370

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2200 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 46..414

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1422..2171

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAAGACCCGG ACAGCTGCGG GAGCGGCGTC CACTTTGGTG ATACC ATG AAT GGC 54

Met Asn Gly

1

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TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC GGT AGC GGC 102  
 Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly Gly Ser Gly  
           5                          10                          15

GGC TGG CAG CCT GAG GCG GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC 150  
 Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe Ala Leu Ile  
           20                          25                          30                          35

TTC CTC GTG GGC ACC GTG GGC AAC GCG CTG GTG CTG GCG GTG CTG CTG 198  
 Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala Val Leu Leu  
                                   40                          45                          50

CGC GGC GGC CAG GCG GTC AGC ACC ACC AAC CTG TTC ATC CTC AAC CTG 246  
 Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu  
                           55                          60                          65

GGC GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCT TTC CAG GCC 294  
 Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro Phe Gln Ala  
           70                          75                          80

ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG CTC TGC AAG 342  
 Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu Leu Cys Lys  
           85                          90                          95

GCT GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC ACG 390  
 Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser Ser Phe Thr  
           100                          105                          110                          115

CTG GCC GCC GTC TCC CTG GAC AGG TGAGTGAACA TCGGAGAACT ATTGTATCTG 444  
 Leu Ala Ala Val Ser Leu Asp Arg  
                                   120

AGATAGGGGCTTGGGCTGGAGTCACTACACAGGGGATCCAGAAGGCATGAGCAGAATGGG 504

CGAGAACACTGAAATTACAAAGTGGCCTGAGGCCGTGAACGCAAGGGGGAGGGAGATTA 564

AGACTCAGTGACTGAGAGTGTCTAAGTCGATGGGAGAAATCGGGTCTCTGGGGTCTCTCGC 624

ATTATTACTGCTTGAGTTAAATGTCTCTGTGAAACATTGCAGTTCTCAGGCCAGAGTTGG 684

CAGGAAAAGTAACTCGCCAGTGTTTCAGATGCTGTTTGAGAGCTGCAGAGAAGCATCTGCT 744

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TCTTAGCACCAAGCTCAGCACCTGGGGCGTTGTCCGGCGCCTTAGGCTTAGGACTGGGCT 804  
 GTGCTGTGTTAAGACCCATGCTCAAGTCCAACGGAGTGTAAGCGAGGGCTCCTAGCTGAC 864  
 ACCCAGAGCCCTCCAGGCCAAGGCTCCCCTCACCAGATGCCAGCGGTTTTATGCTCCTT 924  
 CCATAGGTAAAGGACCCAGAAAGAAACATCCAGTATGCCCGGAGGGATCTTGACTGGAAA 984  
 AGACTGAATCCTGGTCTGGTGACCTTAGTTCCTGCCCTTTCACATCACTTGGACATTCC 1044  
 CACAGAAGAGCGGTGAAGAGGCGGTGGTCCTTATTCTCCTCTGGTTTCCACTGAGTGCAA 1104  
 CATGTGCGTCCTGAGTACGCTGGAGGGACTCACAAAATTTAGCTTTCTT TAGGAGTTTC 1164  
 CTTGCTGTAGTTTGACCCAAGTCTTCTCCAGGTTTCTGTGAGAACCTCAG GCATGAGGGA 1224  
 TCTGCCTCCCCTGGTTGTACCAGAGGATAACAATCACTGCCCCCAGAAATCCAGACAGA 1284  
 TTCTACAACTTTTAGTCTTCGGTGTTTTGGGGGTGCCCTTCACGTGGAG TAGGTCGGTG 1344  
 GCCACATTCCCAGGAGTGACAATAGCCTAGCAGTGAATCCTCTCGCTTAG CTGATGCCCC 1404  
 CCCACTGTCCCCACAGG TAT CTG GCC ATC CGC TAC CCG CTG CAC TCC CGA 1454  
 Tyr Leu Ala Ile Arg Tyr Pro Leu His Ser Arg  
 1 5 10  
 GAG TTG CGC ACA CCT CGA AAC GCG CTG GCC GCC ATC GGG CTC ATC TGG 1502  
 Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp  
 15 20 25  
 GGG CTA GCA CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC CGT CAG 1550  
 Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr Leu Ser Tyr Tyr Arg Gln  
 30 35 40  
 TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC CCA GCA TGG AGC GCA CCT 1598  
 Ser Gln Leu Ala Asn Leu Thr Val Cys His Pro Ala Trp Ser Ala Pro  
 45 50 55  
 CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC GTC TTT AGC TAC CTG CTG 1646  
 Arg Arg Arg Ala Met Asp Leu Cys Thr Phe Val Phe Ser Tyr Leu Leu  
 60 65 70 75

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CCA GTG CTA GTC CTC AGT CTG ACC TAT GCG CGT ACC CTG CGC TAC CTC 1694  
 Pro Val Leu Val Leu Ser Leu Thr Tyr Ala Arg Thr Leu Arg Tyr Leu  
 80 85 90

TGG CGC ACA GTC GAC CCG GTG ACT GCA GGC TCA GGT TCC CAG CGC GCC 1742  
 Trp Arg Thr Val Asp Pro Val Thr Ala Gly Ser Gly Ser Gln Arg Ala  
 95 100 105

AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC GTG GCG GTG CTT TTC TGC 1790  
 Lys Arg Lys Val Thr Arg Met Ile Ile Ile Val Ala Val Leu Phe Cys  
 110 115 120

CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC GTG TGG TTT GGT 1838  
 Leu Cys Trp Met Pro His His Ala Leu Ile Leu Cys Val Trp Phe Gly  
 125 130 135

CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG TTG CGC ATC CTT TCA CAC 1886  
 Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala Leu Arg Ile Leu Ser His  
 140 145 150 155

CTA GTT TCC TAT GCC AAC TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG 1934  
 Leu Val Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile Val Tyr Ala Leu  
 160 165 170

GTC TCC AAG CAT TTC CGT AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG 1982  
 Val Ser Lys His Phe Arg Lys Gly Phe Arg Lys Ile Cys Ala Gly Leu  
 175 180 185

CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC CGA GTG AGC ATC CTG GCG 2030  
 Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly Arg Val Ser Ile Leu Ala  
 190 195 200

CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA CAG GAA TCC ACA GAC CTG 2078  
 Pro Gly Asn His Ser Gly Ser Met Leu Glu Gln Glu Ser Thr Asp Leu  
 205 210 215

ACA CAG GTG AGC GAG GCA GCC GGG CCC CTT GTC CCA CCA CCC GCA CTT 2126  
 Thr Gln Val Ser Glu Ala Ala Gly Pro Leu Val Pro Pro Pro Ala Leu  
 220 225 230 235

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CCC AAC TGC ACA GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT TAA 2171  
 Pro Asn Cys Thr Ala Ser Ser Arg Thr Leu Asp Pro Ala Cys \*  
 240 245 250

AGGACCAAAG GGCATCTAAC AGCTTCTAG 2200

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly  
 1 5 10 15  
 Gly Ser Gly Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe  
 20 25 30  
 Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala  
 35 40 45  
 Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile  
 50 55 60  
 Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro  
 65 70 75 80  
 Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu  
 85 90 95  
 Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser  
 100 105 110  
 Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg  
 115 120



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## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 250 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Tyr Leu Ala Ile Arg Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro  
1 5 10 15

Arg Asn Ala Leu Ala Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu  
20 25 30

Phe Ser Gly Pro Tyr Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn  
35 40 45

Leu Thr Val Cys His Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met  
50 55 60

Asp Leu Cys Thr Phe Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu  
65 70 75 80

Ser Leu Thr Tyr Ala Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp  
85 90 95

Pro Val Thr Ala Gly Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr  
100 105 110

Arg Met Ile Ile Ile Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro  
115 120 125

His His Ala Leu Ile Leu Cys Val Trp Phe Gly Arg Phe Pro Leu Thr  
130 135 140

Arg Ala Thr Tyr Ala Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala  
145 150 155 160

Asn Ser Cys Val Asn Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe

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165	170	175
Arg Lys Gly Phe Arg Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro		
180	185	190
Arg Arg Ala Ser Gly Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser		
195	200	205
Gly Ser Met Leu Glu Gln Glu Ser Thr Asp Leu Thr Gln Val Ser Glu		
210	215	220
Ala Ala Gly Pro Leu Val Pro Pro Pro Ala Leu Pro Asn Cys Thr Ala		
225	230	235
Ser Ser Arg Thr Leu Asp Pro Ala Cys *		
245	250	

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAAGGCTGTT CATTTCCTCA TCTTTC

26

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTGGAGACCA GAGCGTAAAC GATGG

25

(2) INFORMATION FOR SEQ ID NO:14:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGTCGACCCG GTGACTGCAG GCTCAGGTTT CCAGCGCGCC AAACG

45

(2) INFORMATION FOR SEQ ID NO:15:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCCTACCTGA GCTACTACCG TCA

23

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## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ACCAAACCAC ACGCAGAGGA TAAG

24

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCACGATGAG GATCATGCGT GTCACC

26

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TAGGTCAGGC CGAGAACCAG CACAGG

26

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CAGGTAGCTG AAGACGAAGG TGCA

24

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTGCACCTTC GTCTTCAGCT ACCTG

25

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## (2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCTGTGCTGG TTCTCGGCCT GACCTA

26

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TATCTGGCCA TCCGCTACCC GCTGCA

26

## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TTGCGCTACC TCTGGCGCGC CGTCGACCCG GTGGCCGCGG GCTCG

45

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CCAACAATGA CTCCAACCTCT GTGAC

25

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AGGCGCAGAA CTGGTAGGTA TGGAA

25

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## (2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAGCTTCTAG AGATCCCTCG ACCTC

25

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ACGGAATTCTG ACATGAATGG CTCCGGCA

28

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GCTCTAGAGC CCCTTTGGTC CTTTAACAAG CCGG

34

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1365 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 102..1265

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGTCGCACTA GGAGTTGCAG CGGCCGCAGC CCCGGGAGCT TCCCGCTCGC GGAGACCCAG 60

ACGGCTGCAG GAGCCCGGGC AGCCTCGGGG TCAGCGGCAC C ATG AAC GTC TCG 113

Met Asn Val Ser

1

GGC TGC CCA GGG GCC GGG AAC GCG AGC CAG GCG GGC GGC GGG GGA GGC 161

Gly Cys Pro Gly Ala Gly Asn Ala Ser Gln Ala Gly Gly Gly Gly Gly

5

10

15

20

TGG CAC CCC GAG GCG GTC ATC GTG CCC CTG CTC TTC GCG CTC ATC TTC 209

Trp His Pro Glu Ala Val Ile Val Pro Leu Leu Phe Ala Leu Ile Phe

25

30

35

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CTC GTG GGC ACC GTG GGC AAC ACG CTG GTG CTG GCG GTG CTG CTG CGC 257  
 Leu Val Gly Thr Val Gly Asn Thr Leu Val Leu Ala Val Leu Leu Arg  
                     40                    45                    50

GGC GGC CAG GCG GTC AGC ACT ACC AAC CTG TTC ATC CTT AAC CTG GGC 305  
 Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Gly  
                     55                    60                    65

GTG GCC GAC CTG TGT TTC ATC CTG TGC TGC GTG CCC TTC CAG GCC ACC 353  
 Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro Phe Gln Ala Thr  
                     70                    75                    80

ATC TAC ACC CTG GAC GGC TGG GTG TTC GGC TCG CTG CTG TGC AAG GCG 401  
 Ile Tyr Thr Leu Asp Gly Trp Val Phe Gly Ser Leu Leu Cys Lys Ala  
                     85                    90                    95                    100

GTG CAC TTC CTC ATC TTC CTC ACC ATG CAC GCC AGC AGC TTC ACG CTG 449  
 Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser Ser Phe Thr Leu  
                     105                    110                    115

GCC GCC GTC TCC CTG GAC AGG TAT CTG GCC ATC CGC TAC CCG CTG CAC 497  
 Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg Tyr Pro Leu His  
                     120                    125                    130

TCC CGC GAG CTG CGC ACG CCT CGA AAC GCG CTG GCA GCC ATC GGG CTC 545  
 Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala Ala Ile Gly Leu  
                     135                    140                    145

ATC TGG GGG CTG TCG CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC 593  
 Ile Trp Gly Leu Ser Leu Leu Phe Ser Gly Pro Tyr Leu Ser Tyr Tyr  
                     150                    155                    160

CGC CAG TCG CAG CTG GCC AAC CTG ACC GTG TGC CAT CCC GCG TGG AGC 641  
 Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His Pro Ala Trp Ser  
                     165                    170                    175                    180

GCC CCT CGC CGC CGC GCC ATG GAC ATC TGC ACC TTC GTC TTC AGC TAC 689  
 Ala Pro Arg Arg Arg Ala Met Asp Ile Cys Thr Phe Val Phe Ser Tyr  
                     185                    190                    195

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CTG CTT CCT GTG CTG GTT CTC GGC CTG ACC TAC GCG CGC ACC TTG CGC 737  
 Leu Leu Pro Val Leu Val Leu Gly Leu Thr Tyr Ala Arg Thr Leu Arg  
 200 205 210

TAC CTC TGG CGC GCC GTC GAC CCG GTG GCC GCG GGC TCG GGT GCC CGG 785  
 Tyr Leu Trp Arg Ala Val Asp Pro Val Ala Ala Gly Ser Gly Ala Arg  
 215 220 225

CGC GCC AAG CGC AAG GTG ACA CGC ATG ATC CTC ATC GTG GCC GCG CTC 833  
 Arg Ala Lys Arg Lys Val<sup>F</sup> Thr Arg Met Ile Leu Ile Val Ala Ala Leu  
 230 235 240

TTC TGC CTC TGC TGG ATG CCC CAC CAC GCG CTC ATC CTC TGC GTG TGG 881  
 Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile Leu Cys Val Trp  
 245 250 255 260

TTC GGC CAG TTC CCG CTC ACG CGC GCC ACT TAT GCG CTT CGC ATC CTC 929  
 Phe Gly Gln Phe Pro Leu Thr Arg Ala Thr Tyr Ala Leu Arg Ile Leu  
 265 270 275

TCG CAC CTG GTC TCC TAC GCC AAC TCC TGC GTC AAC CCC ATC GTT TAC 977  
 Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn Pro Ile Val Tyr  
 280 285 290

GCG CTG GTC TCC AAG CAC TTC CGC AAA GGC TTC CGC ACG ATC TGC GCG 1025  
 Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg Thr Ile Cys Ala  
 295 300 305

GGC CTG CTG GGC CGT GCC CCA GGC CGA GCC TCG GGC CGT GTG TGC GCT 1073  
 Gly Leu Leu Gly Arg Ala Pro Gly Arg Ala Ser Gly Arg Val Cys Ala  
 310 315 320

GCC GCG CGG GGC ACC CAC AGT GGC AGC GTG TTG GAG CGC GAG TCC AGC 1121  
 Ala Ala Arg Gly Thr His Ser Gly Ser Val Leu Glu Arg Glu Ser Ser  
 325 330 335 340

GAC CTG TTG CAC ATG AGC GAG GCG GCG GGG GCC CTT CGT CCC TGC CCC 1169  
 Asp Leu Leu His Met Ser Glu Ala Ala Gly Ala Leu Arg Pro Cys Pro  
 345 350 355

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GGC GCT TCC CAG CCA TGC ATC CTC GAG CCC TGT CCT GGC CCG TCC TGG 1217  
 Gly Ala Ser Gln Pro Cys Ile Leu Glu Pro Cys Pro Gly Pro Ser Trp  
 360 365 370

CAG GGC CCA AAG GCA GGC GAC AGC ATC CTG ACG GTT GAT GTG GCC TGA 1265  
 Gln Gly Pro Lys Ala Gly Asp Ser Ile Leu Thr Val Asp Val Ala \*  
 375 380 385

AAGCACTTAG CGGGCGCGCTGGGATGTCACAGAGTTGGAGTCATTGTTGGGGGACCGTGG 1325

GGAGAGCTTTGCCTGTTAATAAAACGCACAAACCATTTCA 1365

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 388 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Met Asn Val Ser Gly Cys Pro Gly Ala Gly Asn Ala Ser Gln Ala Gly  
 1 5 10 15

Gly Gly Gly Gly Trp His Pro Glu Ala Val Ile Val Pro Leu Leu Phe  
 20 25 30

Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Thr Leu Val Leu Ala  
 35 40 45

Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile  
 50 55 60

Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro  
 65 70 75 80

Phe Gln Ala Thr Ile Tyr Thr Leu Asp Gly Trp Val Phe Gly Ser Leu  
 85 90 95

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Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser  
 100 105 110

Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg  
 115 120 125

Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala  
 130 135 140

Ala Ile Gly Leu Ile Trp Gly Leu Ser Leu Leu Phe Ser Gly Pro Tyr  
 145 150 155 160

Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His  
 165 170 175

Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Ile Cys Thr Phe  
 180 185 190

Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Gly Leu Thr Tyr Ala  
 195 200 205

Arg Thr Leu Arg Tyr Leu Trp Arg Ala Val Asp Pro Val Ala Ala Gly  
 210 215 220

Ser Gly Ala Arg Arg Ala Lys Arg Lys Val Thr Arg Met Ile Leu Ile  
 225 230 235 240

Val Ala Ala Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile  
 245 250 255

Leu Cys Val Trp Phe Gly Gln Phe Pro Leu Thr Arg Ala Thr Tyr Ala  
 260 265 270

Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn  
 275 280 285

Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg  
 290 295 300

Thr Ile Cys Ala Gly Leu Leu Gly Arg Ala Pro Gly Arg Ala Ser Gly  
 305 310 315 320

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Arg Val Cys Ala Ala Ala Arg Gly Thr His Ser Gly Ser Val Leu Glu  
325 330 335

Arg Glu Ser Ser Asp Leu Leu His Met Ser Glu Ala Ala Gly Ala Leu  
340 345 350

Arg Pro Cys Pro Gly Ala Ser Gln Pro Cys Ile Leu Glu Pro Cys Pro  
355 360 365

Gly Pro Ser Trp Gln Gly Pro Lys Ala Gly Asp Ser Ile Leu Thr Val  
370 375 380

Asp Val Ala \*  
385

## (2) INFORMATION FOR SEQ ID NO:31:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1384 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTGAGCCAGCGCCTTGGCCTCCCTGGGAGATGGGCATCCACGCGGGGGATGGAGCGGGAG 60  
GCGGGACTGGGGACCAAGAAGGGACGCGCAGAGTGGGACAGGACACTAAG AAGGCAGTGG 120  
AAGACAAGCGGGCGCGGAGGAGGAAAAAGAGGAATAAGAATGGGGGACCG TGGTGTCCCT 180  
CGGTTAGATGCGTCCTGGGGCCTGGAAGCCTGGAGAATGTGGCTCTCCAGCGCCGCCCGT 240  
GCCTGACAACGCGCAGCGTTTCCCAGTACGACGCGTTTGTGCGCGTTCATCTCGCTTGAG 300  
CTTAATGCCCTCCGTGAGGGTGGGATAGGACAAAGTGCCCAATATACAGA AGAGTTGAGT 360  
TCCTAAGTAACTCGCTCAGAGTCGCCAGCCAGGGATCGGGTGCGTGAAGTGACCGTCTGT 420

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CTCCTGCAGCCAACTTCAGGCGCCTCCACTGCGCTCGCCTCCAAGCCACGGTTTGGTTGG	480
TTGGTGCAGCTGGCTCAGGTCCAGGCTGTGGATCTTGGGTCCTTTGCAAGGATCCACTCC	540
GGAGTCCCAGCGAGCGTGCCTAAAGGTCCCTAGCTCAGTCCCAGCCCCTCTGCCTCTCG	600
CCTCCAAACAAAACAAAACAAAATAAAAATCCAAAACAAGTCGGGGCCGGGAGAGGAGCGT	660
GCCCTGGGGTTCTTCCTCCCCAGCCAGAGGAGAGCGAGAGACGCACATTTCGGGAGAGCGC	720
GGGACTCAGGTGGAGCTTGAAAGGACACTGGGATGGTTCTGGGGAGGAAATCCGGGTAT	780
TTCCCCTCTCCATCCTCTGGAAAAACAGAGAGGCGAGGCCAGACTGCCCCCACACCTCCT	840
GTAGCCACTGAGCGCGAAGTGC GTTGGTTCCGAGCGCGCTGGTGGGATCCACAAAGCTCG	900
CATTCTCTCAGGAATCCCCTGAGAAATTAAGTGTCCCTTGCCCAACATGTCTTCTCCAGG	960
CTGTCTGCTAGAGCCTCAGGCGCCTCCGCCCTCCCTCCCGCGGCACCGTCACCAGTGGGT	1020
AGTCACAGCCTCCCGGAGCCCATAGCCGGTTCTCCAACCTTTAGTCTTCAGTGGCTTTGG	1080
GGTGCCCTCTCAGTGGAGACTGTGGTTGCAGTCCCCGGGGGCAGCGGGAGAATGGCTTGA	1140
AGGCACACCTTTCTGCTGCCGGGCCCCGCCCATTTCCAGGCTCCGCTGAGTGTCTGGGA	1200
CACGCTGGGAGGCCCCCACCTCCGCCCTCACGCCGAGCCTCACCCCCACCTCCTCTGTGT	1260
GCGGTGTAACCATGCGCTAAGGACCTTCCTCGAGAGCAGCCTTGGGACCGAGGTGCAGGG	1320
GTCGCGGCCCTCCAGCATGAATGTGCCCCGCTCAGCCGACGTCTCCCTTCCCGGTCTGACC	1380
GCAG	1384

## (2) INFORMATION FOR SEQ ID NO:32:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 346 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met Glu Leu Ala Pro Val Asn Leu Ser Glu Gly Asn Gly Ser Asp Pro  
1 5 10 15

Glu Pro Pro Ala Glu Pro Arg Pro Leu Phe Gly Ile Gly Val Glu Asn  
20 25 30

Phe Ile Thr Leu Val Val Phe Gly Leu Ile Phe Ala Met Gly Val Leu  
35 40 45

Gly Asn Ser Leu Val Ile Thr Val Leu Ala Arg Ser Lys Pro Gly Lys  
50 55 60

Pro Arg Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Ser Ile Ala Asp  
65 70 75 80

Leu Ala Tyr Leu Leu Phe Cys Ile Pro Phe Gln Ala Thr Val Tyr Ala  
85 90 95

Leu Pro Thr Trp Val Leu Gly Ala Phe Ile Cys Lys Phe Ile His Tyr  
100 105 110

Phe Phe Thr Val Ser Met Leu Val Ser Ile Phe Thr Leu Ala Ala Met  
115 120 125

Ser Val Asp Arg Tyr Val Ala Ile Val His Ser Arg Arg Ser Ser Ser  
130 135 140



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Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Phe Ile Trp Ala  
145 150 155 160

Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr Tyr Gln Arg Leu Phe  
165 170 175

His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu His Trp Pro Asn Gln  
180 185 190

Leu His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe Gly Tyr Leu  
195 200 205

Leu Pro Leu Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val Leu Asn His  
210 215 220

Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu Ala Ser Lys  
225 230 235 240

Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Phe Gly Ile  
245 250 255

Ser Trp Leu Pro His His Val Ile His Leu Trp Ala Glu Phe Gly Ala  
260 265 270

Phe Pro Leu Thr Pro Ala Ser Phe Phe Phe Arg Ile Thr Ala His Cys  
275 280 285

Leu Ala Tyr Ser Asn Ser Ser Val Asn Pro Ile Ile Tyr Ala Phe Leu  
290 295 300

Ser Glu Asn Phe Arg Lys Ala Tyr Lys Gln Val Phe Lys Cys Arg Val  
305 310 315 320

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Cys Asn Glu Ser Pro His Gly Asp Ala Lys Glu Lys Asn Arg Ile Asp  
325 330 335

Thr Pro Pro Ser Thr Asn Cys Thr His Val  
340 345

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What is claimed is:

1. A nucleic acid encoding a GALR2 receptor.
- 5 2. The nucleic acid of claim 1, wherein the nucleic acid is DNA.
3. The DNA of claim 2, wherein the DNA is cDNA.
- 10 4. The DNA of claim 2, wherein the DNA is genomic DNA.
5. The nucleic acid of claim 1, wherein the nucleic acid is RNA.
- 15 6. The nucleic acid of claim 1, wherein the nucleic acid encodes a vertebrate GALR2 receptor.
7. The nucleic acid of claim 1, wherein the nucleic acid encodes a mammalian GALR2 receptor.
- 20 8. The nucleic acid of claim 1, wherein the nucleic acid encodes a rat GALR2 receptor.
9. The nucleic acid of claim 1, wherein the nucleic acid encodes a human GALR2 receptor.
- 25 10. The nucleic acid of claim 7, wherein the nucleic acid encodes a receptor characterized by an amino acid sequence in the transmembrane region which has a homology of 60% or higher to the amino acid sequence in the transmembrane region of the rat GALR2 receptor and a homology of less than 60% to the amino acid sequence in the transmembrane region of any GALR1 receptor.
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- 35

- 5 11. The nucleic acid of claim 7, wherein the nucleic acid encodes a mammalian GALR2 receptor which has substantially the same amino acid sequence as does the GALR2 receptor encoded by the plasmid K985 (ATCC Accession No. 97426).
- 10 12. The nucleic acid of claim 8, wherein the nucleic acid encodes a rat GALR2 receptor which has an amino acid sequence encoded by the plasmid K985 (ATCC Accession No. 97426).
- 15 13. The nucleic acid of claim 7, wherein the nucleic acid encodes a mammalian GALR2 receptor which has substantially the same amino acid sequence as does the GALR2 receptor encoded by the plasmid K1045 (ATCC Accession No. 97778).
- 20 14. The nucleic acid of claim 8, wherein the nucleic acid encodes a rat GALR2 receptor which has an amino acid sequence encoded by the plasmid K1045 (ATCC Accession No. 97778).
- 25 15. The nucleic acid of claim 8, wherein the nucleic acid encodes a rat GALR2 receptor which has substantially the same amino acid sequence as that shown in Figure 2.
- 30 16. The nucleic acid of claim 8, wherein the nucleic acid encodes a rat GALR2 receptor which has the amino acid sequence shown in Figure 2.
- 35 17. The nucleic acid of claim 7, wherein the nucleic acid encodes a mammalian GALR2 receptor which has substantially the same amino acid sequence as does the GALR2 receptor encoded by the plasmid B029 (ATCC

Accession No. 97735).

- 5 18. The nucleic acid of claim 9, wherein the nucleic acid encodes a human GALR2 receptor which has an amino acid sequence encoded by the plasmid B029 (ATCC Accession No. 97735).
- 10 19. The nucleic acid of claim 7, wherein the nucleic acid encodes a mammalian GALR2 receptor which has substantially the same amino acid sequence as does the GALR2 receptor encoded by the plasmid B039 (ATCC Accession No. \_\_\_\_\_).
- 15 20. The nucleic acid of claim 9, wherein the nucleic acid encodes a human GALR2 receptor which has an amino acid sequence encoded by the plasmid B039 (ATCC Accession No. \_\_\_\_\_).
- 20 21. The nucleic acid of claim 9, wherein the nucleic acid encodes a human GALR2 receptor which has substantially the same amino acid sequence as that shown in Figure 11.
- 25 22. The nucleic acid of claim 9, wherein the nucleic acid encodes a human GALR2 receptor which has the amino acid sequence shown in Figure 11.
- 30 23. An isolated nucleic acid encoding a modified GALR2 receptor, which differs from a GALR2 receptor by having an amino acid(s) deletion, replacement or addition in the third intracellular domain.
- 35 24. The nucleic acid of claim 23, wherein the modified GALR2 receptor differs from a GALR2 receptor by having a deletion in the third intracellular domain.

25. The nucleic acid of claim 23, wherein the modified GALR2 receptor differs from a GALR2 receptor by having a replacement or addition in the third intracellular domain.
- 5 26. A purified GALR2 receptor protein.
27. A vector comprising the nucleic acid of claim 1.
- 10 28. A vector comprising the nucleic acid of either of claims 8 or 9.
- 15 29. A vector of claim 27 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a GALR2 receptor as to permit expression thereof.
- 20 30. A vector of claim 27 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a GALR2 receptor as to permit expression thereof.
- 25 31. A vector of claim 27 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the GALR2 receptor as to permit expression thereof.
- 30 32. A vector of claim 31 which is a baculovirus.
- 35

- 5 33. A vector of claim 27 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding a GALR2 receptor as to permit expression thereof.
- 10 34. A vector of claim 27 wherein the vector is a plasmid.
35. The plasmid of claim 34 designated K985 (ATCC Accession No. 97426).
- 15 36. The plasmid of claim 34 designated K1045 (ATCC Accession No. 97778).
37. The plasmid of claim 34 designated B029 (ATCC Accession No. 97735).
- 20 38. The plasmid of claim 34 designated B039 (ATCC Accession No. \_\_\_\_\_).
39. A cell comprising the vector of claim 27.
- 25 40. A cell of claim 39, wherein the cell is a non-mammalian cell.
- 30 41. A cell of claim 40, wherein the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell.
42. A cell of claim 39, wherein the cell is a mammalian cell.
- 35 43. A mammalian cell of claim 42, wherein the cell is a

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COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell or a CHO cell.

- 5 44. The LM(tk-) cell of claim 43 designated L-rGALR2-8 (ATCC Accession No. CRL-12074).
45. The LM(tk-) cell of claim 43 designated L-rGALR2I-4 (ATCC Accession No. CRL-12223).
- 10 46. The CHO cell of claim 43 designated C-rGalR2-79 (ATCC Accession No. \_\_\_\_\_).
47. An insect cell comprising the vector of claim 31.
- 15 48. An insect cell of claim 47, wherein the insect cell is an Sf9 cell.
49. An insect cell of claim 47, wherein the insect cell is an Sf21 cell.
- 20 50. A membrane preparation isolated from the cell of either of claims 39 or 47.
- 25 51. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence corresponding to a sequence present within one of the two strands of the nucleic acid encoding the
- 30 GALR2 receptor contained in plasmid K985.
- 35 52. A nucleic acid probe comprising at least 15 nucleotides, which probe specifically hybridizes with a nucleic acid encoding a GALR2 receptor, wherein the probe has a unique sequence



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corresponding to a sequence present within one of the two strands of the nucleic acid encoding the GALR2 receptor contained in plasmid K1045.

- 5 53. A nucleic acid probe comprising at least 15  
nucleotides, which probe specifically hybridizes  
with a nucleic acid encoding a GALR2 receptor,  
wherein the probe has a unique sequence  
10 corresponding to a sequence present within (a) the  
nucleic acid sequence shown in Figure 1 or (b) the  
reverse complement of the nucleic acid sequence  
shown in Figure 1.
- 15 54. A nucleic acid probe comprising at least 15  
nucleotides, which probe specifically hybridizes  
with a nucleic acid encoding a GALR2 receptor,  
wherein the probe has a unique sequence  
corresponding to a sequence present within one of  
the two strands of the nucleic acid encoding the  
20 GALR2 receptor contained in plasmid B029.
- 25 55. A nucleic acid probe comprising at least 15  
nucleotides, which probe specifically hybridizes  
with a nucleic acid encoding a GALR2 receptor,  
wherein the probe has a unique sequence  
corresponding to a sequence present within one of  
the two strands of the nucleic acid encoding the  
GALR2 receptor contained in plasmid B039.
- 30 56. A nucleic acid probe comprising at least 15  
nucleotides, which probe specifically hybridizes  
with a nucleic acid encoding a GALR2 receptor,  
wherein the probe has a unique sequence  
corresponding to a sequence present within (a) the  
35 nucleic acid sequence shown in Figure 10 or (b) the

reverse complement of the nucleic acid sequence shown in Figure 10.

57. The nucleic acid probe of any one of claims 51, 52,  
5 53, 54, 55 or 56, wherein the nucleic acid is DNA.
58. The nucleic acid probe of any one of claims 51, 52,  
53, 54, 55 or 56 wherein the nucleic acid is RNA.
- 10 59. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor.
- 15 60. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique fragment of the sequence of a nucleic acid molecule encoding a GALR2 receptor.
- 20 61. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the mRNA of claim 5, so as to prevent translation of the mRNA.
- 25 62. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 4.
- 30 63. An antisense oligonucleotide of either of claims 61 or 62, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.
- 35 64. An antibody capable of binding to a GALR2 receptor

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encoded by the nucleic acid of claim 1.

65. The antibody of claim 64, wherein the GALR2 receptor is a human GALR2 receptor.

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66. An antibody capable of competitively inhibiting the binding of the antibody of claim 64 to a GALR2 receptor.

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67. An antibody of claim 64, wherein the antibody is a monoclonal antibody.

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68. A monoclonal antibody of claim 67 directed to an epitope of a GALR2 receptor present on the surface of a GALR2 receptor expressing cell.

20

69. A pharmaceutical composition comprising an amount of the oligonucleotide of claim 61 capable of passing through a cell membrane effective to reduce expression of a GALR2 receptor and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

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70. A pharmaceutical composition of claim 69, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

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71. A pharmaceutical composition of claim 70, wherein the substance which inactivates mRNA is a ribozyme.

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72. A pharmaceutical composition of claim 69, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by the cells after binding to the structure.

- 5 73. A pharmaceutical composition of claim 72, wherein the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.
- 10 74. A pharmaceutical composition which comprises an amount of the antibody of claim 64 effective to block binding of a ligand to the GALR2 receptor and a pharmaceutically acceptable carrier.
- 15 75. A transgenic nonhuman mammal expressing DNA encoding a GALR2 receptor of claim 1.
- 20 76. A transgenic nonhuman mammal comprising a homologous recombination knockout of the native GALR2 receptor.
- 25 77. A transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a GALR2 receptor of claim 1 so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a GALR2 receptor and which hybridizes to mRNA encoding a GALR2 receptor, thereby reducing its translation.
- 30 78. The transgenic nonhuman mammal of claim 75 or 76, wherein the DNA encoding a GALR2 receptor additionally comprises an inducible promoter.
- 35 79. The transgenic nonhuman mammal of claim 75 or 77, wherein the DNA encoding a GALR2 receptor additionally comprises tissue specific regulatory elements.
80. A transgenic nonhuman mammal of any one of claims 75, 76 or 77, wherein the transgenic nonhuman mammal

is a mouse.

5 81. A process for identifying a chemical compound which specifically binds to a GALR2 receptor which comprises contacting cells containing DNA encoding and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with the compound under conditions suitable for binding, and detecting  
10 specific binding of the chemical compound to the GALR2 receptor.

15 82. A process for identifying a chemical compound which specifically binds to a GALR2 receptor which comprises contacting a membrane fraction from a cell extract of cells containing DNA encoding and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with the compound under conditions  
20 suitable for binding, and detecting specific binding of the chemical compound to the GALR2 receptor.

25 83. The process of claim 81 or 82, wherein the GALR2 receptor is a mammalian GALR2 receptor.

30 84. The process of claim 81 or 82, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid K985 (ATCC Accession No. 97426).

85. The process of claim 81 or 82, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 2 (Seq. ID No. 8).

35 86. The process of claim 81 or 82, wherein the GALR2

receptor has substantially the same amino acid sequence as encoded by the plasmid B029 (ATCC Accession No. 97735).

5      87. The process of claim 81 or 82, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 11 (Seq. ID No. 30).

10     88. The method of any one of claims 81, 82, 83, 84, 85, 86 or 87, wherein the compound is not previously known to bind to a GALR2 receptor.

15     89. A compound determined by the method of claim 88.

20     90. A process for determining whether a chemical compound is a GALR2 receptor agonist which comprises contacting cells transfected with and expressing DNA encoding the GALR2 receptor with the compound under conditions permitting the activation of the GALR2 receptor, and detecting an increase in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor agonist.

25     91. A process for determining whether a chemical compound is a GALR2 receptor agonist which comprises preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, 30     contacting the membrane fraction with the compound under conditions permitting the activation of the GALR2 receptor, and detecting an increase in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor agonist.

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92. The process of claim 90 or 91, wherein the GALR2 receptor is a mammalian GALR2 receptor.

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93. The process of claim 90 or 91, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid K985 (ATCC Accession No. 97426).

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94. The process of claim 90 or 91, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 2 (Seq. ID No. 8).

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95. The process of claim 90 or 91, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid BO29 (ATCC Accession No. 97735).

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96. The process of claim 90 or 91, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 11 (Seq. ID No. 30).

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97. A process for determining whether a chemical compound is a GALR2 receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding the GALR2 receptor with the compound in the presence of a known GALR2 receptor agonist, under conditions permitting the activation of the GALR2 receptor, and detecting a decrease in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor antagonist.

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98. A process for determining whether a chemical compound is a GALR2 receptor antagonist which comprises preparing a cell extract from cells

transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known GALR2 receptor agonist, under conditions permitting the activation of the GALR2 receptor, and detecting a decrease in GALR2 receptor activity, so as to thereby determine whether the compound is a GALR2 receptor antagonist.

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99. The process of claim 97 or 98, wherein the GALR2 receptor is a mammalian GALR2 receptor.

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100. The process of claim 97 or 98, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid K985 (ATCC Accession No. 97426).

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101. The process of claim 97 or 98, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 2 (Seq. ID No. 8).

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102. The process of claim 97 or 98, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid B029 (ATCC Accession No. 97735).

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103. The process of claim 97 or 98, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 11 (Seq. ID No. 30).

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104. The process of any one of claims 81, 82, 83, 84, 85, 86, 87, 88, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102 or 103, wherein the cell is an insect



cell.

5 105. The process of any one of claims 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102 or 103, wherein the cell is a mammalian cell.

10 106. The process of claim 105, wherein the cell is nonneuronal in origin.

107. The process of claim 106, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

15 108. The process of claim 106, wherein the nonneuronal cell is the LM(tk-) cell designated L-rGALR2-8 (ATCC Accession No. CRL-12074).

20 109. The process of claim 106, wherein the nonneuronal cell is the LM(tk-) cell designated L-rGALR2I-4 (ATCC Accession No. CRL-12223).

25 110. The process of claim 106, wherein the nonneuronal cell is the CHO cell designated C-rGalR2-79 (ATCC Accession No. \_\_\_\_\_).

111. The process of claim 105 wherein the compound is not previously known to bind to a GALR2 receptor.

30 112. A compound determined by the process of claim 111.

35 113. A pharmaceutical composition which comprises an amount of a GALR2 receptor agonist determined by the process of claim 90 or 91 effective to increase activity of a GALR2 receptor and a pharmaceutically

acceptable carrier.

114. A pharmaceutical composition of claim 113, wherein the GALR2 receptor agonist is not previously known.

115. A pharmaceutical composition which comprises an amount of a GALR2 receptor antagonist determined by the process of claim 97 or 98 effective to reduce activity of a GALR2 receptor and a pharmaceutically acceptable carrier.

116. A pharmaceutical composition of claim 115, wherein the GALR2 receptor antagonist is not previously known.

117. A process involving competitive binding for identifying a chemical compound which specifically binds to a GALR2 receptor which comprises separately contacting cells expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the GALR2 receptor, a decrease in the binding of the second chemical compound to the GALR2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR2 receptor.

118. A process involving competitive binding for identifying a chemical compound which specifically binds to a human GALR2 receptor which comprises

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separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the GALR2 receptor, a decrease in the binding of the second chemical compound to the GALR2 receptor in the presence of the chemical compound indicating that the chemical compound binds to the GALR2 receptor.

119. A process for determining whether a chemical compound specifically binds to and activates a GALR2 receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with the chemical compound under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GALR2 receptor.

120. A process for determining whether a chemical compound specifically binds to and activates a GALR2 receptor, which comprises contacting a membrane fraction isolated from a cell extract of cells producing a second messenger response and expressing on their cell surface the GALR2 receptor,

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wherein such cells do not normally express the GALR2 receptor, with the chemical compound under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the GALR2 receptor.

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121. A process for determining whether a chemical compound specifically binds to and inhibits activation of a GALR2 receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to activate the GALR2 receptor, and with only the second chemical compound, under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GALR2 receptor.

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122. A process for determining whether a chemical compound specifically binds to and inhibits activation of a GALR2 receptor, which comprises separately contacting a membrane fraction from a

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cell extract of cells producing a second messenger response and expressing on their cell surface the GALR2 receptor, wherein such cells do not normally express the GALR2 receptor, with both the chemical compound and a second chemical compound known to activate the GALR2 receptor, and with only the second chemical compound, under conditions suitable for activation of the GALR2 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the GALR2 receptor.

123. The process of claim 119 or 120, wherein the second messenger response comprises arachidonic acid release and the change in second messenger response is an increase in arachidonic acid levels.

124. The process of claim 119 or 120, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger response is an increase in intracellular calcium levels.

125. The process of claim 119 or 120, wherein the second messenger response comprises inositol phospholipid hydrolysis and the change in second messenger response is an increase in inositol phospholipid hydrolysis.

126. The process of claim 121 or 122, wherein the second

5 messenger response comprises arachidonic acid release and the change in second messenger response is a smaller increase in the level of arachidonic acid in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

10 127. The process of claim 121 or 122, wherein the second messenger response comprises intracellular calcium levels, and the change in second messenger response is a smaller increase in the intracellular calcium levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

15 128. The process of claim 121 or 122, wherein the second messenger response comprises inositol phospholipid hydrolysis, and the change in second messenger response is a smaller increase in inositol phospholipid hydrolysis in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

20 129. A process of any one of claims 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127 or 128, wherein the GALR2 receptor is a mammalian GALR2 receptor.

25 130. The process of claim 129, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid K985 ATCC Accession No. 97426).

30 131. The process of claim 129, wherein the GALR2 receptor has substantially the same amino acid sequence as

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that shown in Figure 2 (Seq. ID No. 8).

5 132. The process of claim 129, wherein the GALR2 receptor has substantially the same amino acid sequence as encoded by the plasmid B029 (ATCC Accession No. 97735).

10 133. The process of claim 129, wherein the GALR2 receptor has substantially the same amino acid sequence as that shown in Figure 11 (Seq. ID No. 30).

15 134. The process of any one of claims 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132 or 133, wherein the cell is an insect cell.

20 135. The process of any one of claims 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132 or 133, wherein the cell is a mammalian cell.

25 136. The process of claim 135, wherein the mammalian cell is nonneuronal in origin.

30 137. The process of claim 136, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

35 138. The process of claim 136, wherein the nonneuronal cell is the LM(tk-) cell designated L-rGALR2-8 (ATCC Accession No. CRL-12074).

139. The process of claim 136, wherein the nonneuronal cell is the LM(tk-) cell designated L-rGALR2I-4 (ATCC Accession No. CRL-12223).

140. The process of claim 136, wherein the nonneuronal cell is the CHO cell designated C-rGalR2-79 (ATCC Accession No. \_\_\_\_\_).
- 5 141. The process of claim 135, wherein the compound is not previously known to bind to a GALR2 receptor.
142. A compound determined by the process of claim 141.
- 10 143. A pharmaceutical composition which comprises an amount of a GALR2 receptor agonist determined by the process of any one of claims 119, 120, 123, 124, or 125 effective to increase activity of a GALR2 receptor and a pharmaceutically acceptable carrier.
- 15 144. A pharmaceutical composition of claim 143, wherein the GALR2 receptor agonist is not previously known.
- 20 145. A pharmaceutical composition which comprises an amount of a GALR2 receptor antagonist determined by the process of any one of claims 121, 122, 126, 127 or 128 effective to reduce activity of a GALR2 receptor and a pharmaceutically acceptable carrier.
- 25 146. A pharmaceutical composition of claim 145, wherein the GALR2 receptor antagonist is not previously known.
- 30 147. A method of screening a plurality of chemical compounds not known to bind to a GALR2 receptor to identify a compound which specifically binds to the GALR2 receptor, which comprises
- 35 (a) contacting cells transfected with and expressing DNA encoding the GALR2 receptor with



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a compound known to bind specifically to the GALR2 receptor;

- 5 (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the GALR2 receptor, under conditions permitting binding of compounds known to bind the GALR2 receptor;
- 10 (c) determining whether the binding of the compound known to bind to the GALR2 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so
- 15 (d) separately determining the binding to the GALR2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds
- 20 to the GALR2 receptor.

148. A method of screening a plurality of chemical compounds not known to bind to a GALR2 receptor to identify a compound which specifically binds to the

25 GALR2 receptor, which comprises

- 30 (a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a compound known to bind specifically to the GALR2 receptor;
- 35 (b) contacting the preparation of step (a) with the plurality of compounds not known to bind

specifically to the GALR2 receptor, under conditions permitting binding of compounds known to bind the GALR2 receptor;

- 5 (c) determining whether the binding of the compound known to bind to the GALR2 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

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- (d) separately determining the binding to the GALR2 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the GALR2 receptor.

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149. A method of claim 147 or 148, wherein the GALR2 receptor is a mammalian GALR2 receptor.

- 20 150. A method of screening a plurality of chemical compounds not known to activate a GALR2 receptor to identify a compound which activates the GALR2 receptor which comprises

- 25 (a) contacting cells transfected with and expressing the GALR2 receptor with the plurality of compounds not known to activate the GALR2 receptor, under conditions permitting activation of the GALR2 receptor;

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- (b) determining whether the activity of the GALR2 receptor is increased in the presence of the compounds; and if so

- 35 (c) separately determining whether the activation

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of the GALR2 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR2 receptor.

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151. A method of screening a plurality of chemical compounds not known to activate a GALR2 receptor to identify a compound which activates the GALR2 receptor which comprises

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(a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds not known to activate the GALR2 receptor, under conditions permitting activation of the GALR2 receptor;

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(b) determining whether the activity of the GALR2 receptor is increased in the presence of the compounds; and if so

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(c) separately determining whether the activation of the GALR2 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the GALR2 receptor.

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152. A method of claim 150 or 151, wherein the GALR2 receptor is a mammalian GALR2 receptor.

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153. A method of screening a plurality of chemical compounds not known to inhibit the activation of a GALR2 receptor to identify a compound which inhibits

the activation of the GALR2 receptor, which comprises

5 (a) contacting cells transfected with and expressing the GALR2 receptor with the plurality of compounds in the presence of a known GALR2 receptor agonist, under conditions permitting activation of the GALR2 receptor;

10 (b) determining whether the activation of the GALR2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR2 receptor in the absence of the plurality of compounds; and if so

15 (c) separately determining the inhibition of activation of the GALR2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the  
20 GALR2 receptor.

154. A method of screening a plurality of chemical compounds not known to inhibit the activation of a  
25 GALR2 receptor to identify a compound which inhibits the activation of the GALR2 receptor, which comprises

30 (a) preparing a cell extract from cells transfected with and expressing DNA encoding the GALR2 receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the plurality of compounds in the presence of a known GALR2 receptor agonist,  
35 under conditions permitting activation of the

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GALR2 receptor;

5 (b) determining whether the activation of the GALR2 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the GALR2 receptor in the absence of the plurality of compounds; and if so

10 (c) separately determining the inhibition of activation of the GALR2 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the GALR2 receptor.

15 155. A method of any one of claims 90, 91, 97, 98, 150, 151, 153, or 154, wherein activation of the GALR2 receptor is determined by a second messenger assay.

20 156. The method of claim 155, wherein the second messenger is cyclic AMP, intracellular calcium, or an inositol phospholipid.

25 157. A method of claim 153 or 154, wherein the GALR2 receptor is a mammalian GALR2 receptor.

158. A method of any one of claims 147, 148, 150, 151, 153, or 154, wherein the cell is a mammalian cell.

30 159. A method of claim 158, wherein the mammalian cell is non-neuronal in origin.

35 160. The method of claim 159, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk-) cell or an NIH-3T3 cell.

161. A pharmaceutical composition comprising a compound identified by the method of claim 150 and a pharmaceutically acceptable carrier.
- 5 162. A pharmaceutical composition comprising a compound identified by the method of claim 153 and a pharmaceutically acceptable carrier.
- 
- 10 163. A method of detecting expression of a GALR2 receptor by detecting the presence of mRNA coding for the GALR2 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of any one of claims 51, 52, 53, 54, 55, 56 or 60 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the GALR2 receptor by the cell.
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- 20 164. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by the inhibition of a GALR2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of any one of claims 115, 116, or 162 effective to decrease the activity of the GALR2 receptor in the subject, thereby treating the abnormality in the subject.
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- 30 165. The method of claim 164, wherein the abnormality is obesity or bulimia.
- 35 166. A method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a GALR2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of any one of claims

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113, 114, or 161 effective to activate the GALR2 receptor in the subject.

5 167. The method of claim 166, wherein the abnormal condition is anorexia.

168. The method of claim 164 or 166, wherein the compound binds selectively to a GALR2 receptor.

10 169. The method of claim 168, wherein the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR1 receptor.

15 170. The method of claim 168, wherein the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR3 receptor.

20 171. A method of detecting the presence of a GALR2 receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 64 under conditions permitting binding of the antibody to the receptor, detecting the presence of the  
25 antibody bound to the cell, and thereby detecting the presence of a GALR2 receptor on the surface of the cell.

30 172. A method of determining the physiological effects of varying levels of activity of GALR2 receptors which comprises producing a transgenic nonhuman mammal of claim 78 whose levels of GALR2 receptor activity are varied by use of an inducible promoter which regulates GALR2 receptor expression.

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- 5 173. A method of determining the physiological effects of varying levels of activity of GALR2 receptors which comprises producing a panel of transgenic nonhuman mammals of claim 78 each expressing a different amount of GALR2 receptor.
- 10 174. A method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a GALR2 receptor comprising administering a compound to the transgenic nonhuman mammal of any one of claims 75, 15 78, 79, or 80, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overactivity of a GALR2 receptor, the alleviation of the abnormality identifying the compound as an antagonist.
- 20 175. An antagonist identified by the method of claim 174.
176. A pharmaceutical composition comprising an antagonist identified by the method of claim 174 and a pharmaceutically acceptable carrier.
- 25 177. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a GALR2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 176, thereby 30 treating the abnormality.
178. A method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity 35 of a GALR2 receptor comprising administering a



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compound to the transgenic nonhuman mammal of any one of claims 75, 78, 79, or 80, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal, the alleviation of the abnormality identifying the compound as an agonist.

179. An agonist identified by the method of claim 178.

180. A pharmaceutical composition comprising an agonist identified by the method of claim 178 and a pharmaceutically acceptable carrier.

181. A method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a GALR2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 180, thereby treating the abnormality.

182. A method for diagnosing a predisposition to a disorder associated with the activity of a specific human GALR2 receptor allele which comprises:

- a. obtaining DNA of subjects suffering from the disorder;
- b. performing a restriction digest of the DNA with a panel of restriction enzymes;
- c. electrophoretically separating the resulting DNA fragments on a sizing gel;
- d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing

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with a unique sequence included within the sequence of a nucleic acid molecule encoding a human GALR2 receptor and labelled with a detectable marker;

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e. detecting labelled bands which have hybridized to the DNA encoding a human GALR2 receptor of claim 9 labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;

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f. preparing DNA obtained for diagnosis by steps a-e; and

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g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

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183. The method of claim 182, wherein a disorder associated with the activity of a specific human GALR2 receptor allele is diagnosed.

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184. A method of preparing the purified GALR2 receptor of claim 26, which comprises:

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a. inducing cells to express GALR2 receptor;

b. recovering the receptor from the induced cells; and

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c. purifying the receptor so recovered.

185. A method of preparing the purified GALR2 receptor of claim 26, which comprises:

5       a. inserting nucleic acid encoding the GALR2 receptor in a suitable vector;

      b. introducing the resulting vector in a suitable host cell;

10       c. placing the resulting cell in suitable condition permitting the production of the isolated GALR2 receptor;

15       d. recovering the receptor produced by the resulting cell; and

      e. purifying the receptor so recovered.

20   186. A method of modifying feeding behavior of a subject which comprises administering to the subject an amount of a compound which is a GALR2 receptor agonist or antagonist effective to increase or decrease the consumption of food by the subject so  
25       as to thereby modify feeding behavior of the subject.

      187. The method of claim 186, wherein the compound is a GALR2 receptor antagonist and the amount is  
30       effective to decrease the consumption of food by the subject.

      188. The method of claim 186 or 187, wherein the compound is administered in combination with food.

189. The method of claim 186, wherein the compound is a GALR2 receptor agonist and the amount is effective to increase the consumption of food by the subject.

5 190. The method of claim 186 or 189, wherein the compound is administered in combination with food.

191. The method of claim 186 or 189, wherein the compound binds selectively to a GALR2 receptor.

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192. The method of claim 191, wherein the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR1 receptor.

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193. The method of claim 191, wherein the compound binds to the GALR2 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to a GALR3 receptor.

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194. The method of claim 191, wherein the compound binds to the GALR2 receptor with an affinity greater than one hundred-fold higher than the affinity with which the compound binds to a GALR1 receptor.

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195. The method of claim 191, wherein the compound binds to the GALR2 receptor with an affinity greater than one hundred-fold higher than the affinity with which the compound binds to a GALR3 receptor.

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196. The method of claim 186, wherein the subject is a vertebrate, a mammal, a human or a canine.

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197. A method for determining whether a compound is a GALR2 antagonist which comprises:

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(a) administering to an animal a GALR2 agonist and measuring the amount of food intake in the animal;

5 (b) administering to a second animal both the GALR2 agonist and the compound, and measuring the amount of food intake in the second animal; and

10 (c) determining whether the amount of food intake is reduced in the presence of the compound relative to the amount of food intake in the absence of the compound, so as to thereby determine whether the compound is a GALR2 antagonist.

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198. A method of screening a plurality of compounds to identify a compound which is a GALR2 antagonist which comprises:

20 (a) administering to an animal a GALR2 agonist and measuring the amount of food intake in the animal;

25 (b) administering to a second animal the GALR2 agonist and at least one compound of the plurality of compounds and measuring the amount of food intake in the animal;

30 (c) determining whether the amount of food intake is reduced in the presence of at least one compound of the plurality relative to the amount of food intake in the absence of at least one compound of the plurality, and if so;

35 (d) separately determining whether each compound is

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a GALR2 antagonist according to the method of claim 132, so as to thereby identify a compound which is a GALR2 antagonist.

5 199. The method of claim 197 or 198, wherein the GALR2 agonist is [D-Trp]<sub>2</sub>-galanin<sub>(1-29)</sub>.

200. The method of either of claims 197 or 198 wherein the animal is a non-human mammal.

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201. The method of claim 200, wherein the mammal is a rodent.

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202. A process of claim 81 or 82, which further comprises determining whether the compound selectively binds to the GALR2 receptor relative to another galanin receptor.

20

203. The process of claim 202, wherein the determination whether the compound selectively binds to the GALR2 receptor comprises:

25

(a) determining the binding affinity of the compound for the GALR2 receptor and for such other galanin receptor; and

30

(b) comparing the binding affinities so determined, the presence of a higher binding affinity for the GALR2 receptor than for such other galanin receptor indicating that the compound selectively binds to the GALR2 receptor.

204. A process of claim 202, wherein such other galanin receptor is a GALR1 receptor.

35

- 201 -

205. A process of claim 202, wherein such other galanin receptor is a GALR3 receptor.

206. A method of decreasing feeding behavior of a subject which comprises administering a compound which is a GALR2 receptor antagonist and a compound which is a Y5 receptor antagonist, the amount of such antagonists being effective to decrease the feeding behavior of the subject.

207. The method of claim 206, wherein the GALR2 antagonist and the Y5 antagonist are administered in combination.

208. The method of claim 206, wherein the GALR2 antagonist and the Y5 antagonist are administered once.

209. The method of claim 206, wherein the GALR2 antagonist and the Y5 antagonist are administered separately.

210. The method of claim 209, wherein the GALR2 antagonist and the Y5 antagonist are administered once.

211. The method of claim 209, wherein the galanin receptor antagonist is administered for about 1 week to 2 weeks.

212. The method of claim 209, wherein the Y5 receptor antagonist is administered for about 1 week to 2 weeks.

213. The method of claim 209, wherein the GALR2

- 202 -

antagonist and the Y5 antagonist are administered alternately.

5 214. The method of claim 213, wherein the GALR2 antagonist and the Y5 antagonist are administered repeatedly.

10 215. A method of claim 213 or 214, wherein the galanin receptor antagonist is administered for about 1 week to 2 weeks.

15 216. A method of claim 213 or 214, wherein the Y5 receptor antagonist is administered for about 1 week to 2 weeks.

20 217. A method of any one of claims 206, 207, 208, or 209, wherein the compound is administered in a pharmaceutical composition comprising a sustained release formulation.

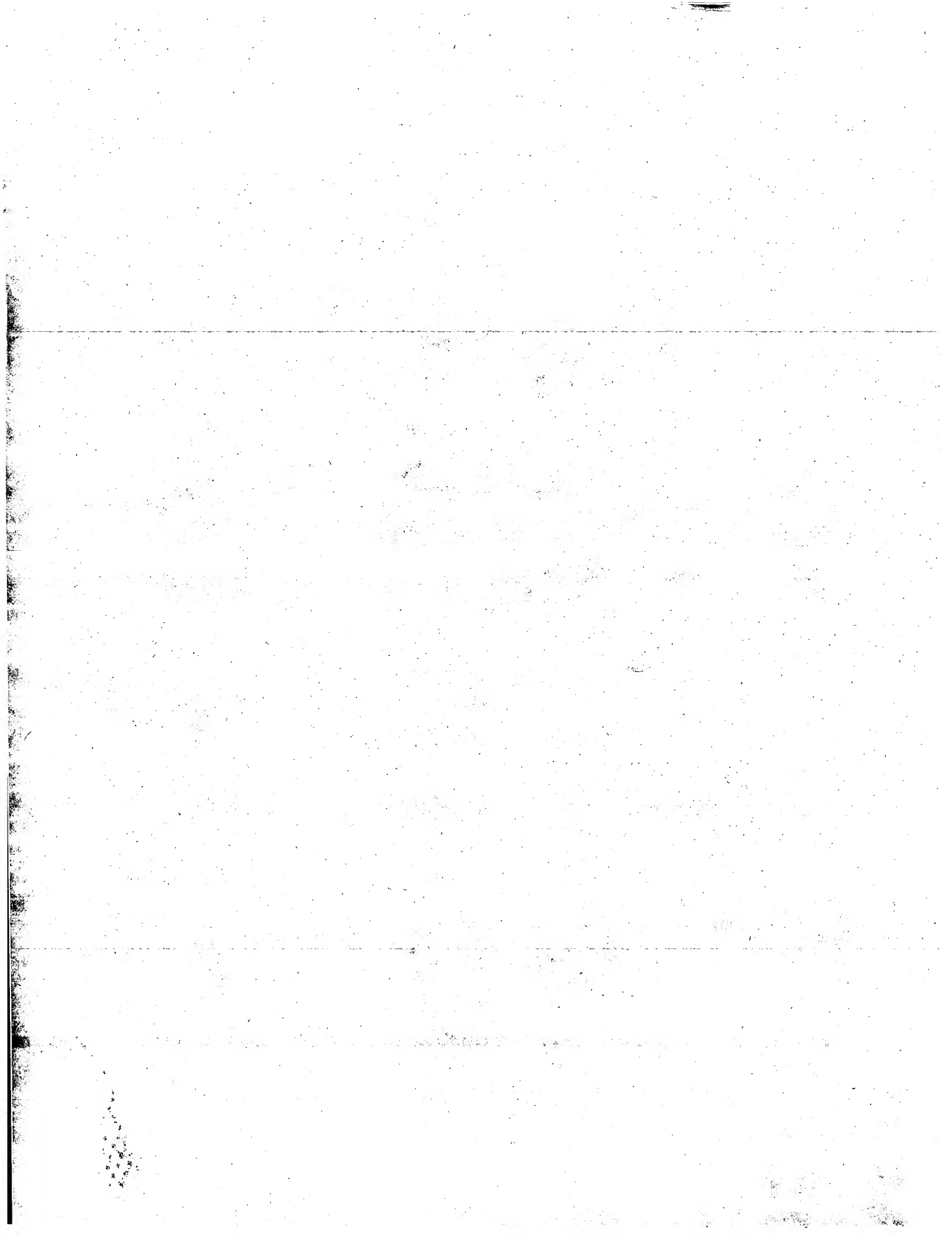
25 218. A method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a GALR2 receptor agonist effective to decrease nociception in the subject.

30 219. A method of treating pain in a subject which comprises administering to the subject an amount of a compound which is a GALR2 receptor agonist effective to treat pain in the subject.

35 220. A method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a GALR2 receptor antagonist effective to treat Alzheimer's disease in



the subject.



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FIGURE 1

1 CAAGACCCGGACAGCTGCGGGAGGCGGTCCACTTTGGTGATACCATGAATGGCTCCGGC 60  
61 AGCCAGGGCGCGGAGAACACGAGCCAGGAAGCGGTAGCGGGCTGGCAGCCTGAGGCG 120  
121 GTCCTTGTAACCCCTATTTTCGCGCTCATCTTCCTCGTGGCACCGTGGGCAACGCGCTG 180  
181 GTGCTGGCGGTGCTGCTGCGCGGCGGCCAGCGGTGAGCACCAACCTGTTTCATCCTC 240  
241 AACCTGGCGGTGGCCGACCTGTGTTTCATCCTGTGCTGCGTGCCTTCCAGGCCACCATC 300  
301 TACACCTGGACGACTGGGTGTCGGCTCGCTGCTCTGCAAGGCTGTTTCATTTCCATC 360  
361 TTTCTCACTATGACGCCAGCAGCTTCACGCTGGCCGCGCTCTCCCTGGACAGTATCTG 420  
421 GCCATCCGCTACCCGCTGCACTCCGAGAGTTGCGCACACCTCGAAACGCGCTGGCCGCC 480  
481 ATCGGGCTCATCTGGGGGTAGCACTGCTCTTCTCCGGGCCCTACCTGAGCTACTACCGT 540  
541 CAGTCGACGCTGGCCCAACCTGACAGTATGCCACCCAGCATGGAGCGCACCTCGACGTCGA 600  
601 GCCATGGACCTCTGCACCTTCGTCTTTAGCTACCTGCTGCCAGTGTAGTCCCTCAGTCTG 660  
661 ACCTATGCGCGTACCCCTGCGCTACCTCTGCGGCACAGTCGACCCGGTACTGCAGGCTCA 720  
721 GGTTCACGCGGCCAAACGCAAGGTGACACGGATGATCATCATCGTGGCGGTGCTTTTC 780  
781 TGCCCTCTGTTGGATGCCCCACCAACGCGCTTATCCTCTGCGTGTGGTTGGTCGCTTCCCG 840  
841 CTCACGCGTGCCCACTTACGCGTTGCGCATCCTTTACACCTAGTTTCCTATGCCAACTCC 900  
901 TGTGTCAACCCCATCGTTTACGCTCTGCTCTCCAAAGCATTTCCGTAAAGGTTTCCGCCAAA 960  
961 ATCTGCGCGGCGCTGCTGCGCCCTGCCCCGAGCGGAGCTTCGGCCGAGTGAGCATCCTG 1020  
1021 GCGCCTGGGAACCATAGTGGCAGCATGCTGGAACAGGAATCCACAGACCTGACACAGGTG 1080  
1081 AGCGAGGACGCGGGCCCTTGTCCCAACCCGCACTTCCCAACTGCACAGCCTCGAGT 1140  
1141 AGAACCCCTGGATCCGGCTTGTAAAGGACCAAGGGCATCTAACAGCTTCTAG 1193



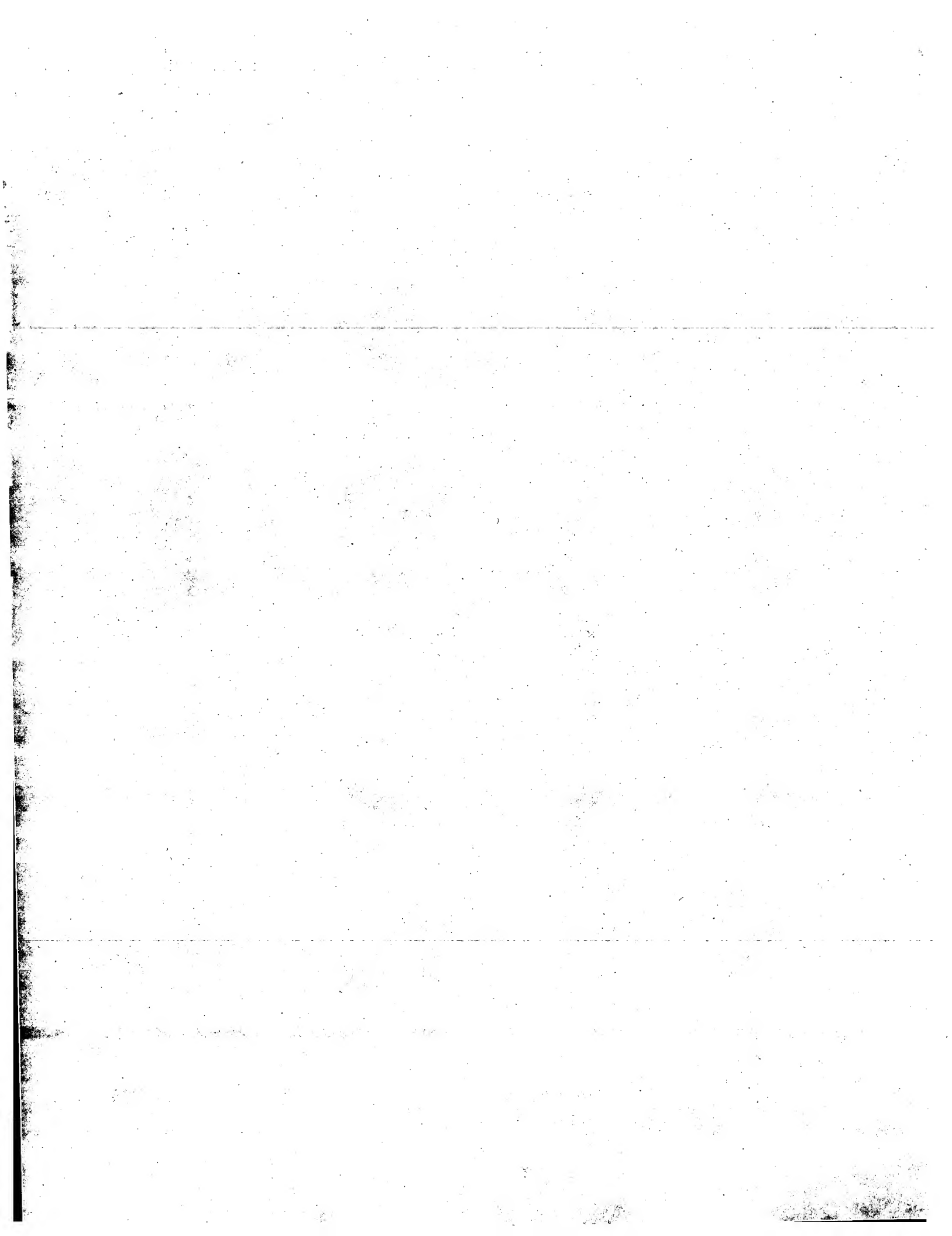
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FIGURE 2

1	M	W	V	N	F	V	L	N	L	A	L	V	V	F	S	K	R	D	C	20
21	N	Q	G	L	Q	H	D	A	S	P	V	T	A	G	Y	G	V	L	T	40
41	G	P	N	F	A	F	R	L	Y	R	L	A	V	R	A	F	S	T	A	60
61	S	E	A	I	T	L	Y	A	Y	R	S	G	L	F	N	R	I	Q	S	80
81	G	A	L	L	I	I	L	A	R	R	L	S	F	P	S	K	L	V	S	100
101	S	V	V	N	Y	F	A	I	Q	A	T	G	C	L	C	I	A	S	R	120
121	Q	L	L	L	T	L	I	G	S	M	Y	S	L	T	V	C	P	E	T	140
141	G	V	A	G	L	T	R	L	Q	D	A	Q	C	R	N	A	G	A	L	160
161	A	P	V	V	D	M	Y	I	L	L	R	R	W	A	P	G	N	A	D	180
181	E	L	L	A	D	H	P	W	A	C	T	A	M	T	I	L	H	G	P	200
201	N	F	L	D	W	A	L	G	N	T	L	K	P	Y	V	L	S	P	A	220
221	T	F	R	L	V	S	H	L	L	F	R	R	H	A	Y	R	G	L	C	240
241	S	A	G	C	F	S	S	A	T	V	Y	K	H	L	A	P	S	V		260
261	Q	L	G	F	G	F	R	L	V	F	L	V	A	R	L	A	M	P		280
281	E	I	Q	I	S	T	E	L	C	S	W	T	L	I	V	P	L	P		300
301	G	F	A	L	L	L	L	F	H	Y	R	R	I	L	S	R	E	P		320
321	G	L	V	C	L	A	R	S	P	L	T	M	L	S	K	R	Q	A		340
341	S	V	S	C	C	A	T	G	A	L	V	I	C	H	H	A	E	L		360
361	G	G	T	V	K	V	P	P	W	P	D	I	V	L	F	S	S	P		372
	G	T	T	P	A	S	R	Y	S	V	P	I	W	V	R	G	T	N		



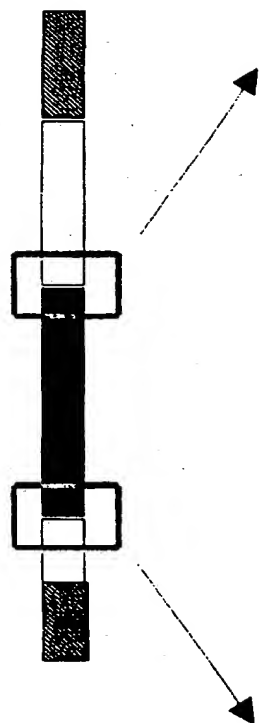






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Figure 3B



TCC CTG GAC **AG/g** tgagtgaacat  
S L D R -----

tgccccccactgtcccaac **ag/G** TAT CTG GCC ATC CGC  
/ Y L A I R  
-----



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**FIGURE 3C**

414 GTGAGTGAACATCGGAGAACTATTGTATCTGAGATAGGGGCTTGGGCTGGAGTCACTACA 473  
474 CAGGGGATCCAGAAGGCATGAGCAGAAATGGCGAGAACACTGAAATTACAAAGTGGCCTG 533  
534 AGGCCGTGAAACGCAAGGGGAGGAGATTAAAGACTCAGTGACTGAGAGTGTCTAAGTCG 593  
594 ATGGGAGAAATCGGGTCTCTGGGTCCTCGCATTTACTGCTTGAGTTAAATGTCTCTG 653  
654 TGAAACATTGCAGTTCTCAGGCCAGAGTTGGCAGGAAAGTAACTCGCCAGTGTTCAGAT 713  
714 GCTGTTTGAGAGCTGCAGAGAAAGCATCTGCTTCTTAGCACCAAGCTCAGCACCTGGGGCG 763  
774 TTGTCCGGCGCCTTAGGCTTAGGACTGGGCTGTGCTGTGTTAAGACCCATGCTCAAGTCC 833  
834 AACGGAGTGTAAAGCGAGGCTCCTAGCTGACACCCAGAGCCCTCCAGGCCAAGGCTCCCC 893  
894 TCACCGAGATGCCAGCGGTTTATGCTCCTTCCATAGGTAAGGACCCAGAAAGAAACAT 953  
954 CCAGTATGCCCCGGAGGGATCTTGACTGGAAAGACTGAATCCTGGTCTGGTGACCTTAGT 1013  
1014 TCCCTGCCCTTTCACATCACTTGGACATTCCACAGAGAGCGGTGAAGAGCGGTGGTC 1073  
1074 CTTATTCTCCTCTGGTTTCCACTGAGTGCAACATGTGCGTCTGAGTACGCTGGAGGGAC 1133  
1134 TCACAAAATTTCAGCTTTTCTTTAGGAGTTTCCCTTGCTGTAGTTTGACCCCAAGTCTTCTCC 1193  
1194 AGGTTTCTGTCAGAAACCTCAGGCATGAGGGATCTGCCTCCCCCTGGTTGTCAACCAGAGGAT 1253  
1254 AACAACTACTGCCCCCAGAAATCCAGACAGATTCTACAACCTTTTAGTCTTCGGTGTTTG 1313  
1314 GGGTGCCCCCTTCACGTGGAGTAGGTCGGTGGCCACATTCCCAGGAGTGACAATAGCCCTA 1373  
1374 GCAGTGAATCCTCTCGCTTAGCTGATGCCCCCCCCCCTGCTCCCCACAG 1420



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FIG. 4A-1



FIG. 4A-2

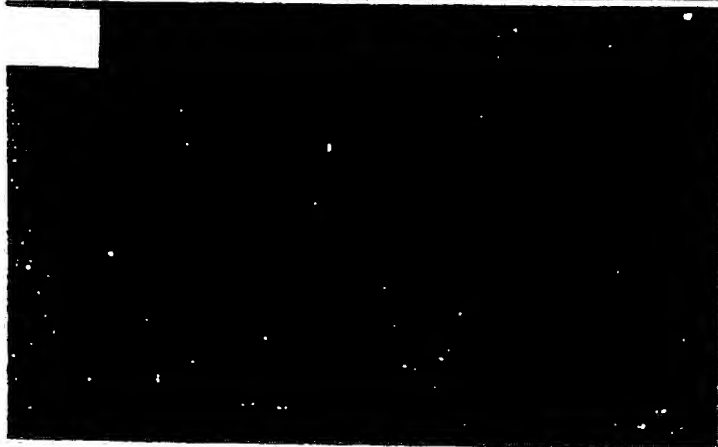
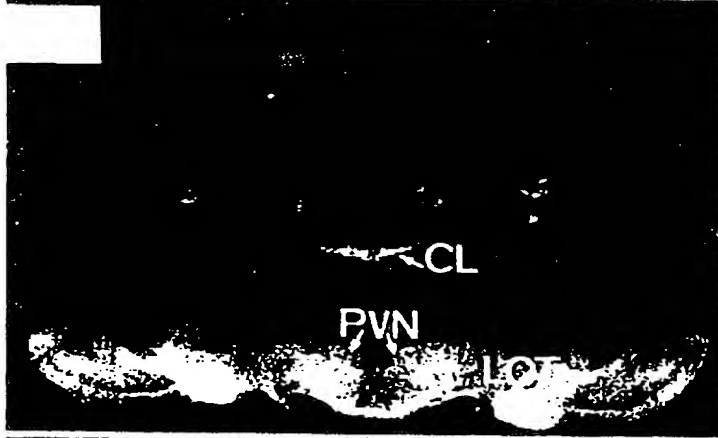


FIG. 4A-3



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FIG. 4A-4

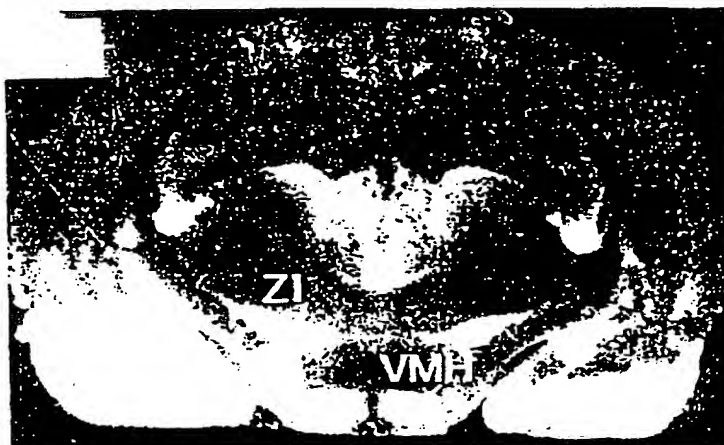


FIG. 4A-5



FIG. 4A-6







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FIG. 4B-2

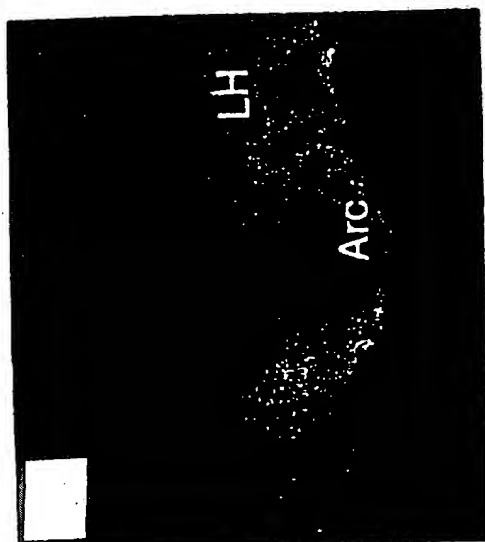


FIG. 4B-1

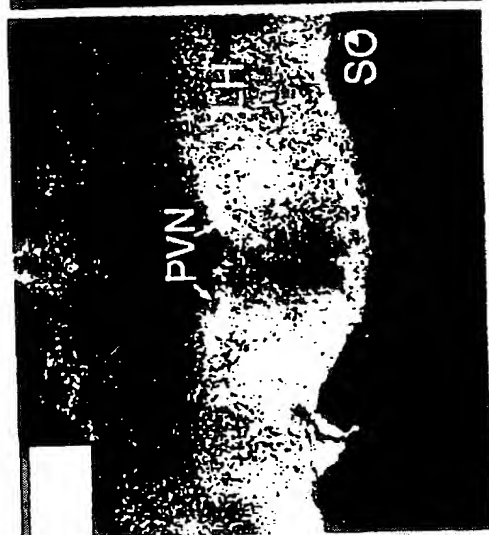


FIG. 4B-4

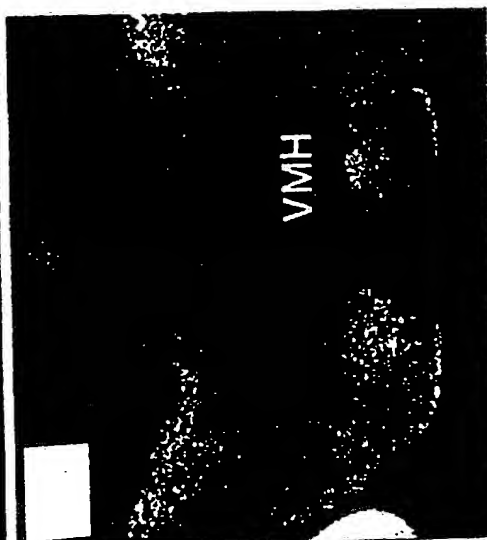
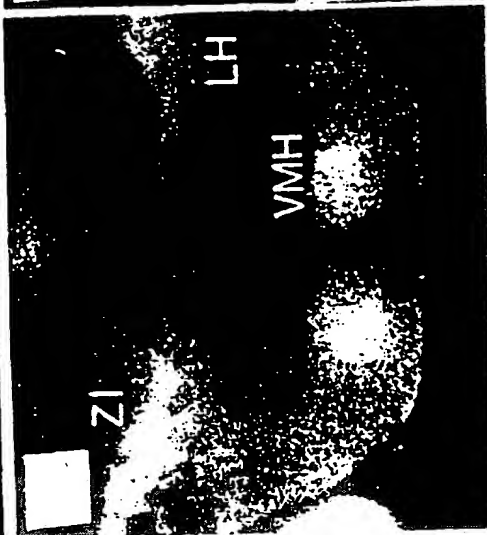


FIG. 4B-3





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FIG. 4B-6

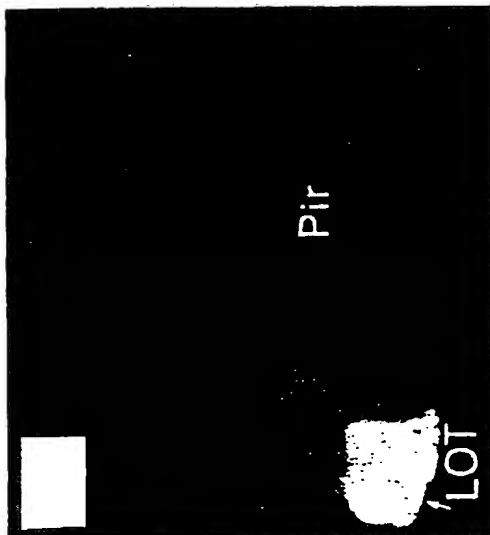


FIG. 4B-5



FIG. 4B-8

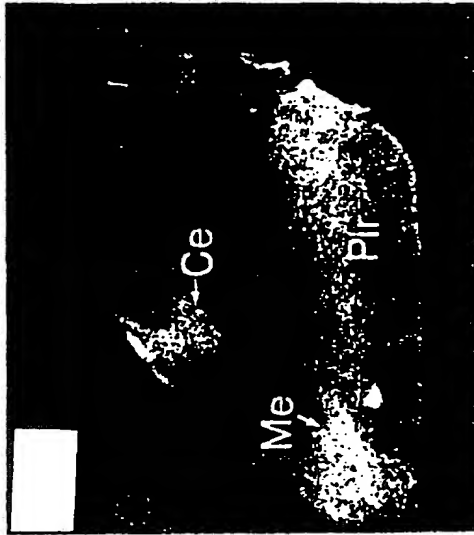


FIG. 4B-7





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FIG. 4C-1



FIG. 4C-2

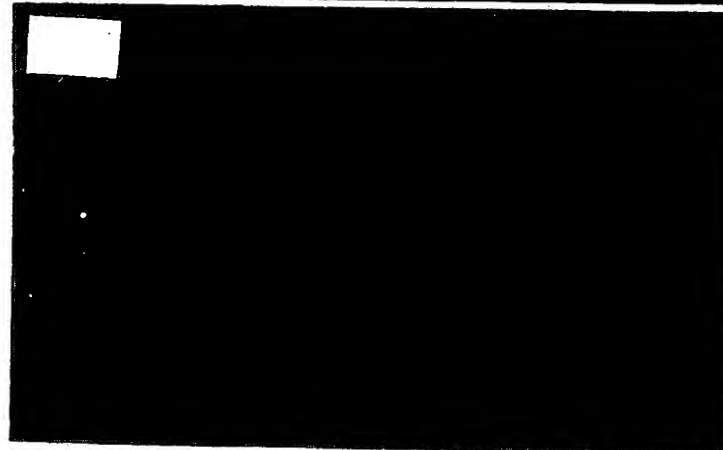


FIG. 4C-3



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FIG. 4C-4



FIG. 4C-5

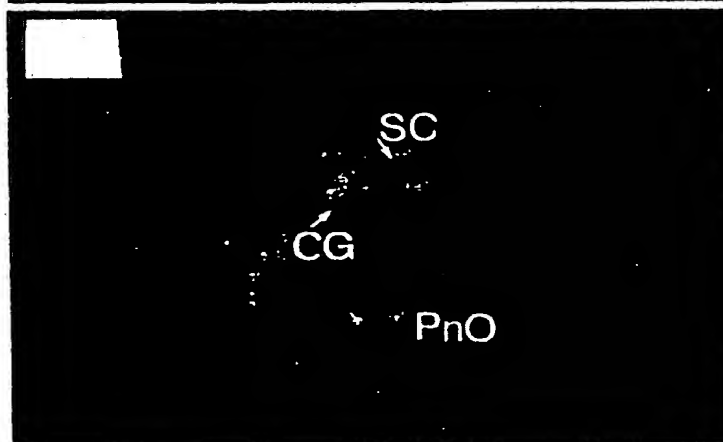
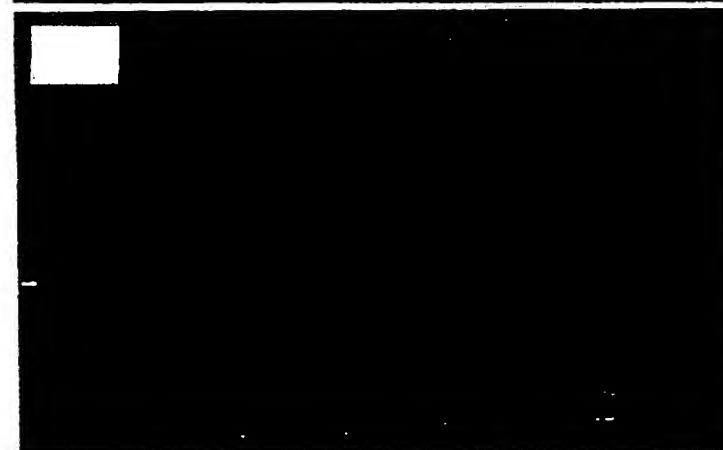


FIG. 4C-6

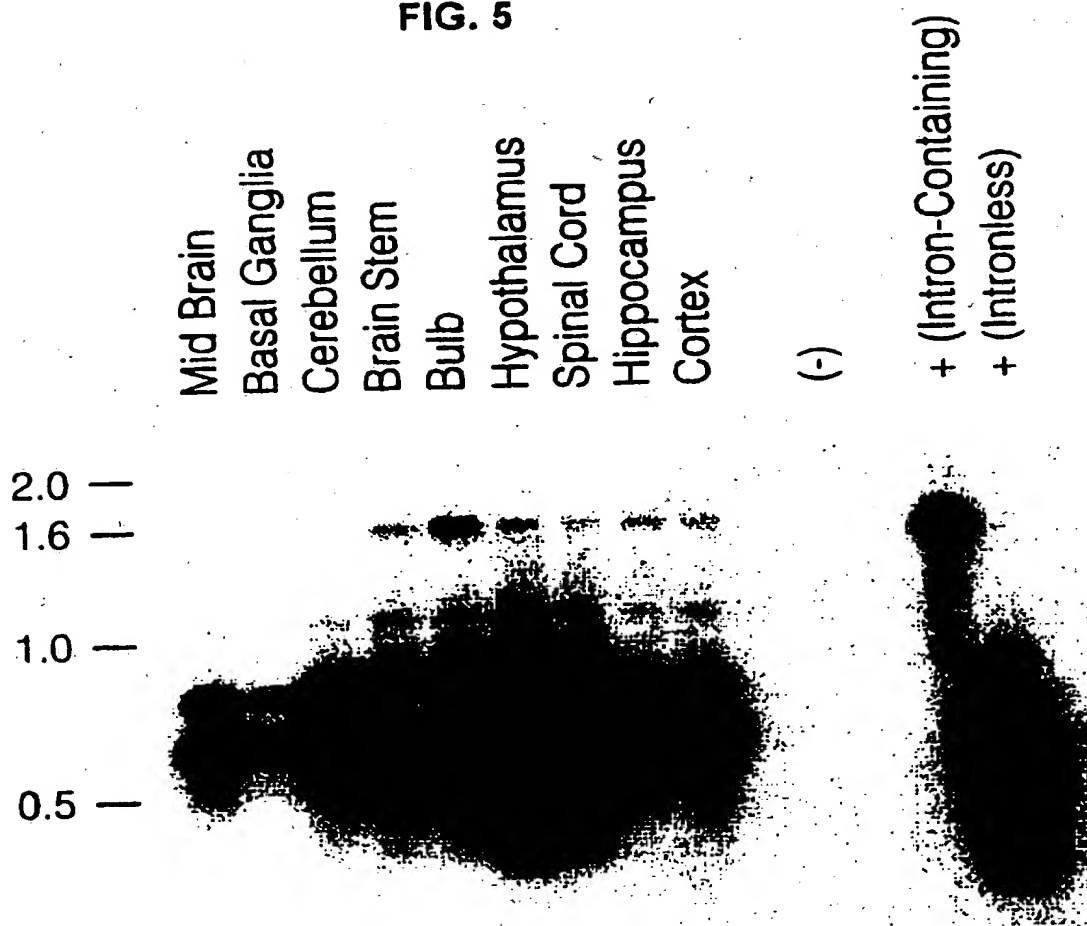






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FIG. 5





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FIG. 6A

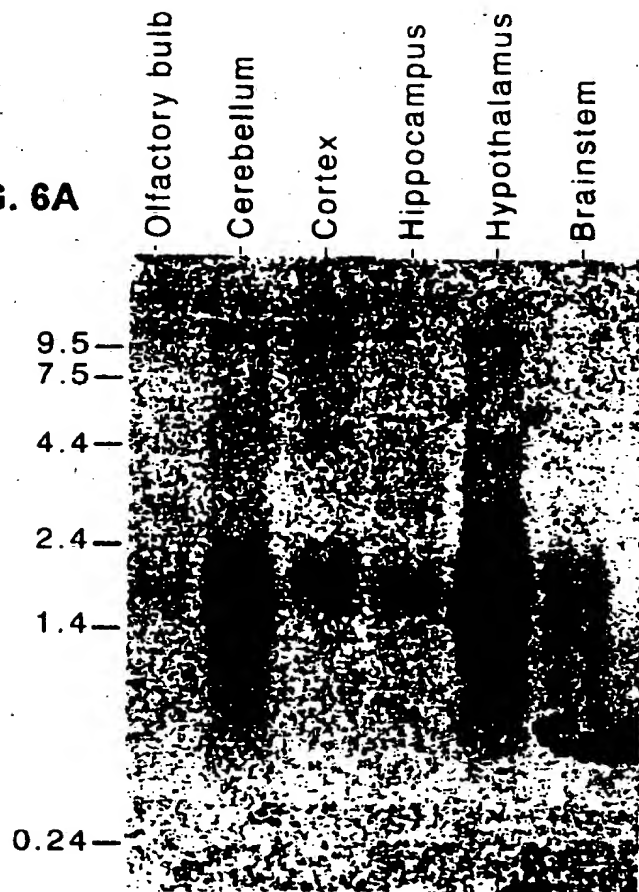


FIG. 6B





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FIG. 7A

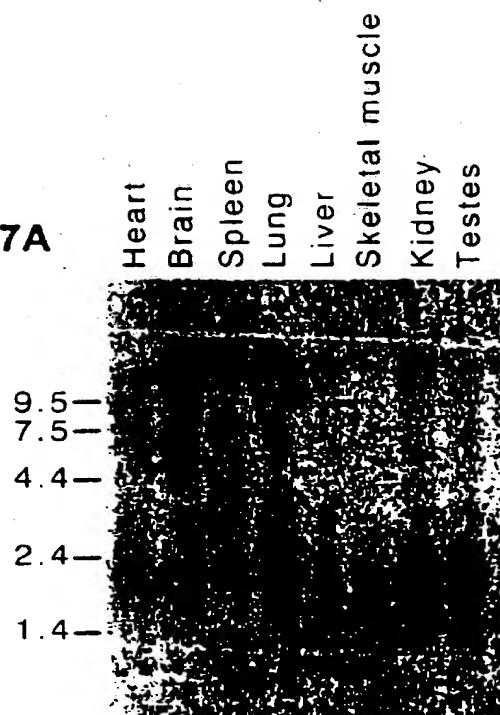


FIG. 7B





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FIG. 8A



FIG. 8B



FIG. 8C

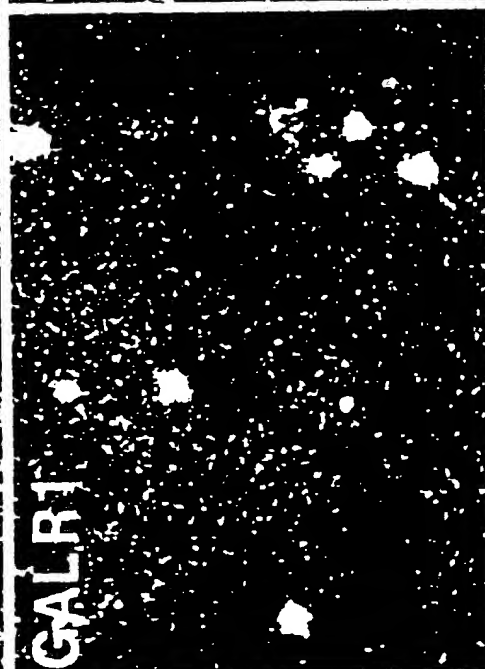
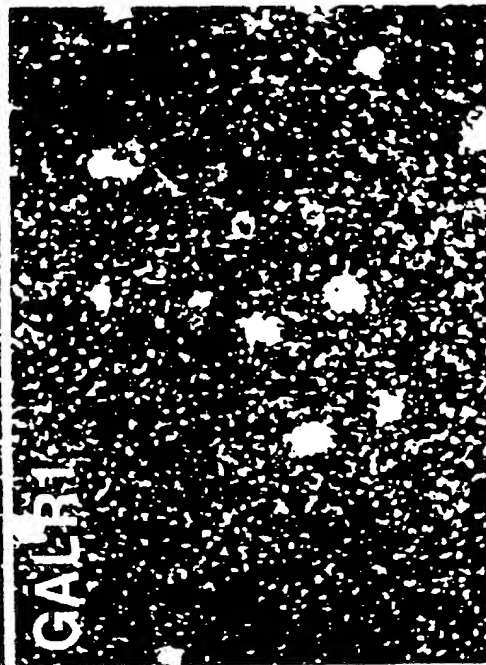


FIG. 8D







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FIG. 9A

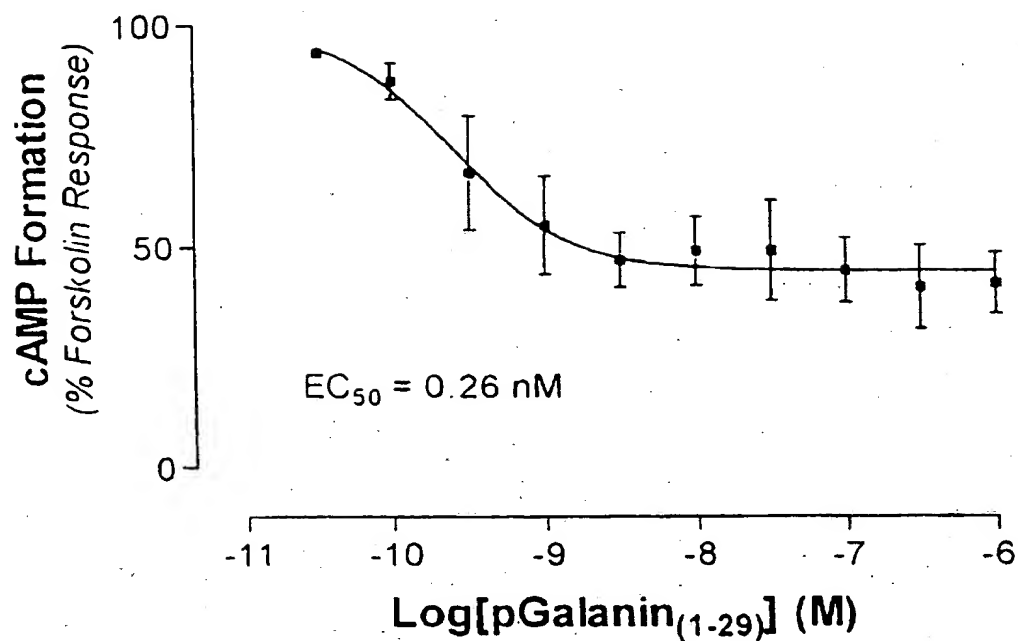
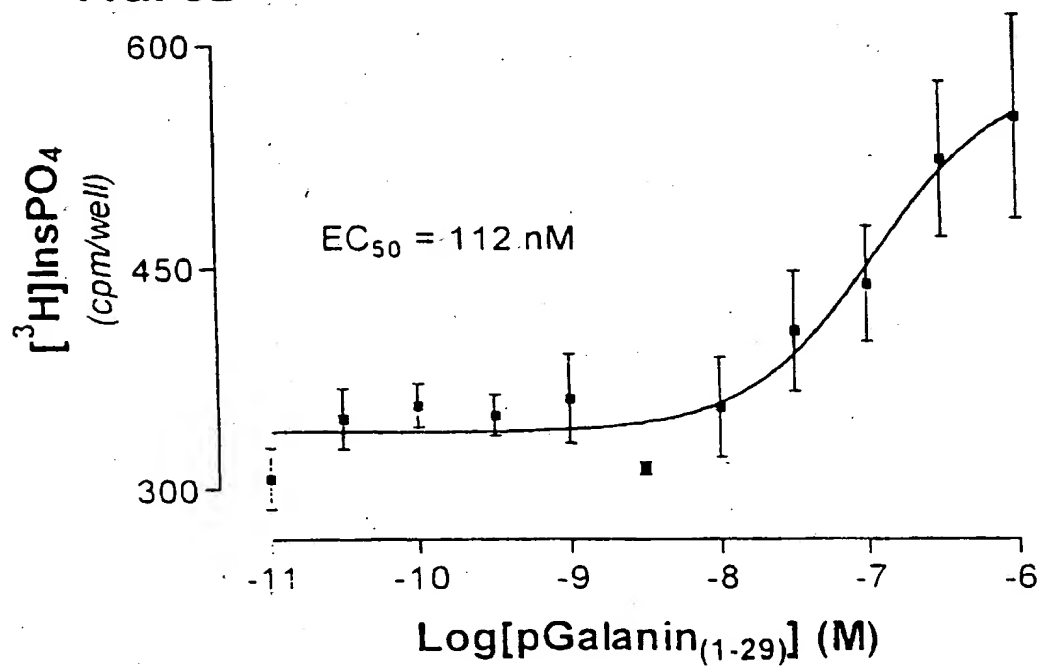


FIG. 9B





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**FIGURE 10**

**FIGURE 10**

-100	AGTCGCAC	TAGGAGTTGCAGCGGGCCAGCCCCGGGAGCTTCCCGCTCGCGGAGACCCAG	-41
-40	ACGGCTG	CAGAGCCCGGAGCCTCGGGTCAGCGCACCAATGAACGTCTCGGGCTGCC	19
20	CAGGGCC	GGAAACGAGCCAGCGGGCGGGGAGGCTGCGACCCCGAGGCGGTCA	79
80	TCGTGCC	CCCTGCTCTTCGCGCTCATCTTCCTCGTGGCACCGTGGGCAACACGCTGTGC	139
140	TGGCGGT	GTGCTGCGCGGGCCAGGCGGTACGACTACCAACCTGTTCACTTAACC	199
200	TGGCGGT	GGCGGCTCGCTGCTGTGCAAGCGGTGACATTCCTCATCTTCC	259
260	CCCTGGA	CGGTGGTTCGGCTCGCTGCTGCAAGCGGTGACATTCCTCATCTTCC	319
320	TCACCAT	GCACGCGAGCTGCGCACGCTCGAAACGCGCTGACCTACTACCGCCAGT	379
380	TCCGTA	CCCCGTGCTGCTTCTCCGGGCCCTACCTAGCTACTACCGCCGCCA	439
440	GGCTCAT	CTGGGGCTGTGCTGCTGCTGCAAGCGGTGACATTCCTCATCTTCC	499
500	CGCAGCT	GGCCAACTGACCTGCTTACGTAACCTGCTTCCCTGCTGCTGCGGTG	559
560	TGGACAT	CTGCACCTTCGCTTACGTAACCTGCTTCCCTGCTGCTGCGGTG	619
620	ACGCGCG	CACCTTACGTAACCTGCTTCCCTGCTGCTGCTGCTGCGGTG	679
680	CCCGCG	CGCCACCGCTCATCTCTGCGTGTGCTTCCCTGCTGCTGCGGTG	739
740	TCTGCT	GGATGCCCTTACGCGCTGGTCTCCAGCACCTTCCGCAAGGCTTCCG	799
800	CGCGGC	CCACTGTTACGCGCTGGTCTCCAGCACCTTCCGCAAGGCTTCCG	859
860	TCAACCC	CCCATCGTTACGCGCTGGTCTCCAGCACCTTCCGCAAGGCTTCCG	919
920	GCGCGG	CCCTGCTGGCCGTGTTGGAGCGCGGAGTCCAGCACCTTCCGCAAGG	979
980	GGGGCA	CCACAGTGGCAGCGTGTGCGGCTGCTGCGCAAGGCTTCCGCAAGG	1039
1040	AGCGCG	CGGCGCTTCCGCGGCTGCTGCGCAAGGCTTCCGCAAGGCTTCCG	1099
1100	GTCC	TGGCCCTTCCGCGGCTGCTGCGCAAGGCTTCCGCAAGGCTTCCG	1159
1160	CCTGA	AAGCACTTAGCGGGCGCTGGGATGTACAGAGTTGGAGTCAATTGT	1219
1220	CGTGGG	GAGAGCTTGGCCCTGTTAATAAAGCACAAACCATTTCA	1264



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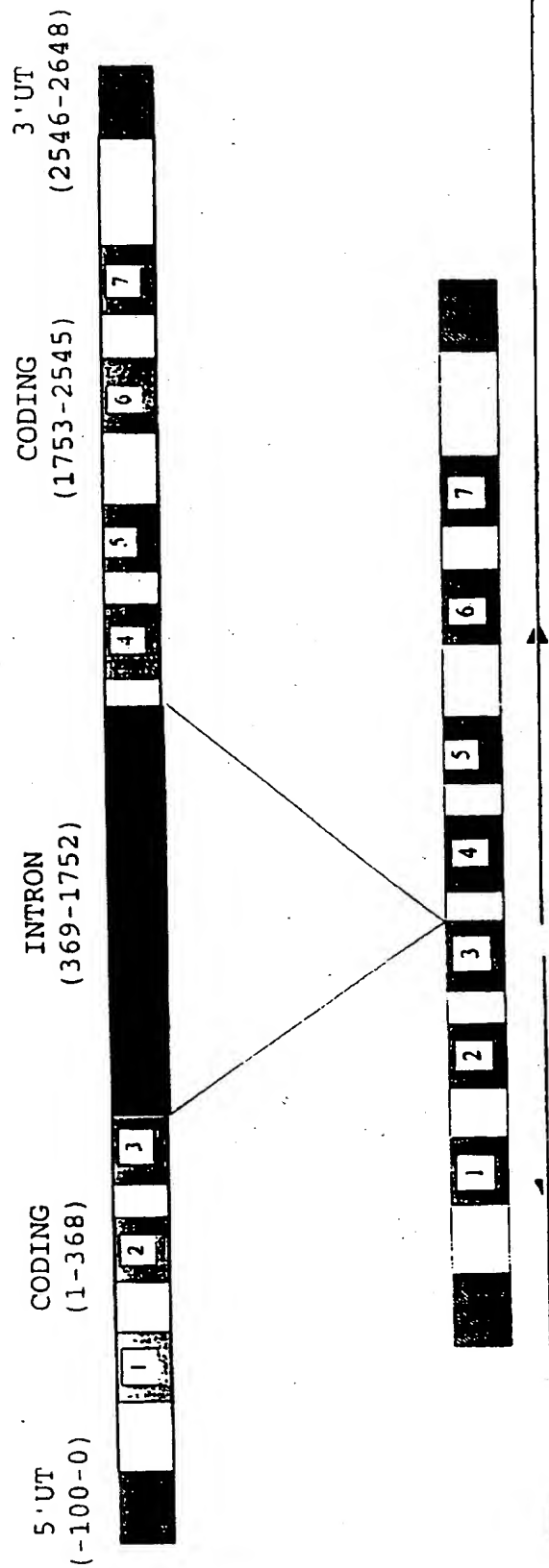
## FIGURE 11

20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320	340	360	380	387
G	T	T	P	A	S	R	Y	S	V	P	I	W	V	R	G	S	Q	S	
G	G	T	V	K	V	P	P	W	P	D	L	V	L	F	S	S	S	D	
G	V	S	C	C	A	T	G	A	L	V	I	C	H	H	A	E	A	G	
G	L	V	C	L	A	R	S	P	L	A	M	L	S	K	R	R	G	A	
G	F	A	L	L	L	L	F	H	Y	R	R	I	L	S	G	E	P	K	
A	I	Q	I	S	T	E	L	C	S	W	T	L	I	V	P	L	C	P	
Q	L	G	F	G	F	R	L	V	F	L	V	A	R	L	A	V	P	G	
S	A	G	C	F	S	S	S	T	V	Y	K	H	L	A	R	S	R	Q	
A	F	R	L	V	S	H	L	L	F	R	R	H	A	Y	G	G	L	W	
N	L	L	D	W	A	L	G	N	T	L	K	P	Y	V	L	S	A	S	
G	L	L	A	G	H	P	W	A	C	T	A	M	T	I	L	H	G	P	
A	P	V	V	D	M	Y	I	L	I	R	R	W	A	P	G	T	A	G	
G	V	A	G	L	T	R	L	Q	D	A	R	C	R	N	A	G	A	P	
P	I	L	L	T	L	I	G	S	M	Y	A	L	T	V	C	R	E	C	A
C	V	V	N	Y	F	A	I	Q	A	T	G	C	L	C	I	A	S	P	V
G	A	L	L	I	I	L	A	R	R	L	S	F	P	S	T	A	M	E	D
S	E	T	I	T	L	Y	A	Y	R	G	G	L	F	N	R	A	H	L	V
V	P	N	F	A	F	R	L	Y	R	L	A	A	Q	A	F	C	L	I	T
N	H	G	L	Q	H	D	A	S	P	V	A	A	G	Y	G	V	L	C	L
M	W	V	N	F	V	L	N	L	A	L	V	V	F	S	K	R	D	P	I
1	21	41	61	81	101	121	141	161	181	201	221	241	261	281	301	321	341	361	381



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Figure 12A

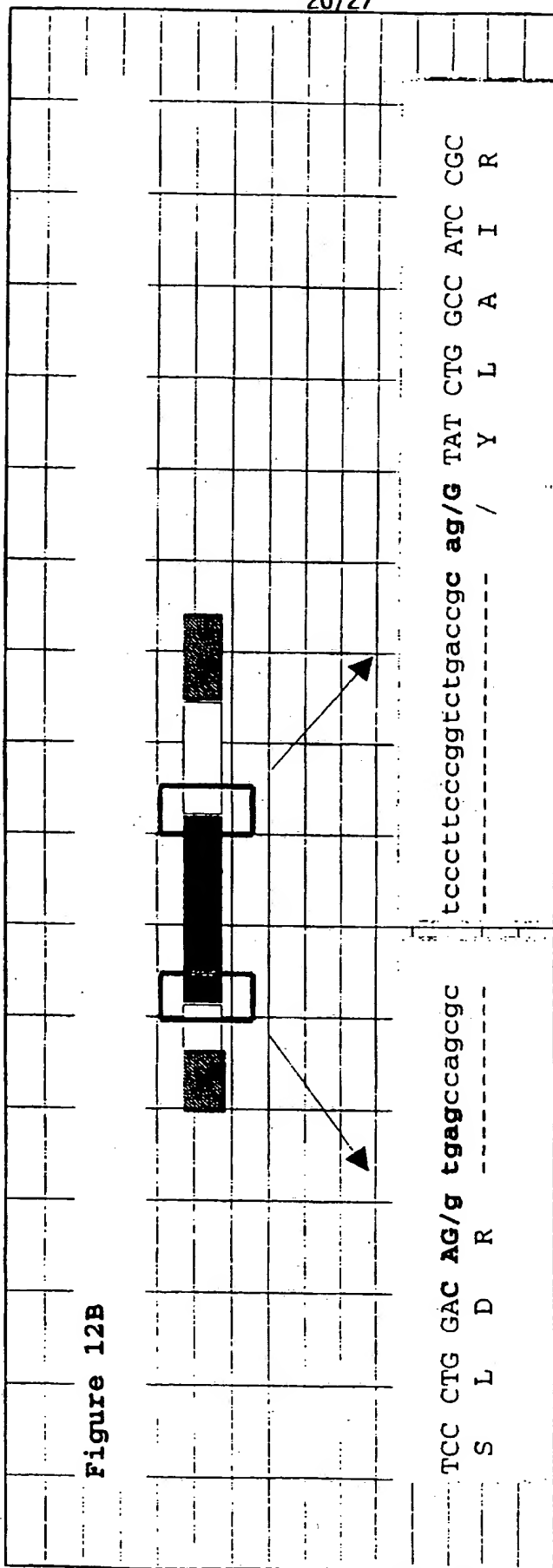






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Figure 12B





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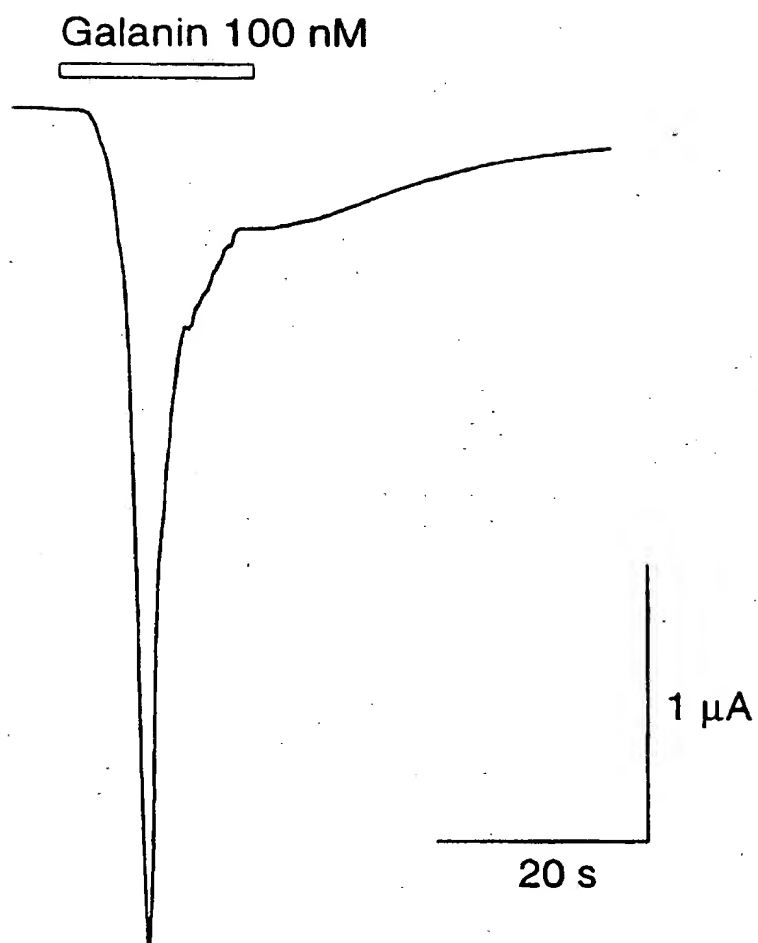
FIGURE 12C

369	GTGAGCCAGCGCCTTGCCCTCCCTGGAGATGGGCATCCACGCGGGGATGGAGCGGGAG	428
429	GCGGACTGGGGACCAAGAAGGACGCGCAGAGTGGACAGGACACTAAGAAGGCAGTGG	488
489	AAGACAAGCGGGCGGAGGAGAAAGAGGAATAAGAAATGGGGACCGTGTGTCCCT	548
549	CGGTAGATGCGTCTGGGCCCTGGAAGCCTGGAGAAATGTGGCTCTCCAGCGCCGCCCGT	608
609	GCCTGACAAACGCGACGCTTCCACGTACGACGCGTTTGTGCGCGTTTCATCTCGCTTGAG	668
669	CTTAATGCCCTCCGTGAGGGTGGGATAGGACAAAGTGCCCCAATATACAGAAGAGTTGAGT	728
729	TCCTAAGTAACTCGCTCAGAGTCGCCAGCCAGGGATCGGGTGCGTGAAGTGACCGTCTGT	788
789	CTCCTGCAGCCAACTTCAGGCGCCTCCACTGCGCTCGCCTCCAAGCCACGGTTTGGTTGG	848
849	TTGGTGCAGCTGGCTCAGGTCCAGGCTGTGGATCTTGGTCTTTCGCAAGGATCCACTCC	908
909	GGAGTCCCAGCGAGCGTGCCTAAAGTCCCTAGCTCAGTCCAGCCACTCTGCCCTCTCG	968
969	CCTCCAAACAAACAAACAAATAAAATCCAAACAAAGTCGGGGCCGGAGAGGAGCGT	1028
1029	GCCCTGGGGTTCTTCCTCCCCAGCCAGAGGAGAGCGAGACGCAATTCGGGAGAGCGC	1088
1089	GGGACTCAGGTGAGCTTGAAAGGACACTGGGATGGTTCTTGGGAGGAAATCCGGGTAT	1148
1149	TTCCCCCTCTCCATCCTCTGGAAACACAGAGAGGCGGAGCCAGACTGCCCCCCACACCTCCT	1208
1209	GTAGCCACTGAGCGCGAAGTGCCTTGGTTCGAGCGCGCTGGTGGGATCCACAAAGCTCG	1268
1269	CATTCTCTCAGGAATCCCCCTGAGAAATTAACGTGTCCTTGCCCAACATGTCTTCTCCAGG	1328
1329	CTGTCTGCTAGAGCCTCAGGCGCTCCGCCCTCCCTCCCGGGCACCGTCACCAGTGGGT	1388
1389	AGTCACAGCCTCCCGAGCCCATAGCCGGTTCTCCAAACCTTTAGTCTTCAGTGGCTTTGG	1448
1449	GGTGCCCTCTCAGTGGAGACTGTGGTTGCAGTCCCGGGGCGAGCGGAGAAATGGCTTGA	1508
1509	AGGCACACCTTTCCTGCTGCCGGGCCGCCCCCATTTCCAGGCTCCGCTGAGTGTCTGGGA	1568
1569	CACGCTGGGAGGCCCCACCTCCGCCCTCACGCCGAGCCTCACCCCCACCTCTCTGTGT	1628
1629	GCGGTGTAACCATGCGCTAAGGACCTTCCTTCGAGAGCAGCCTTGGGACCGAGGTGCAGGG	1688
1689	GTCGCGGCCCTCCAGCATGAATGTGCCCCGCTCAGCCGACGTCTCCCTTCCCCGGTCTGACC	1748
1749	GCAG	1752



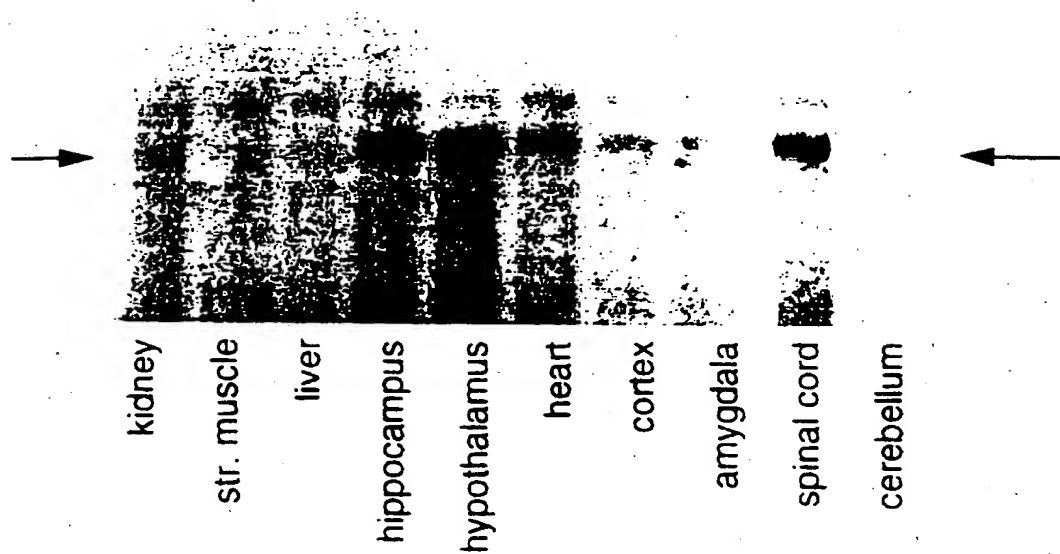
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FIGURE 13





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**FIGURE 14**





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Figure 15A

rGALR2	. . . . .	M N G S G S Q G A E N T S Q E G G S G .	19
hGALR2	. . . . .	M N V S G C P G A G N A S Q A G G G .	19
rGALR1	M E L A P V N L S E G N G S D P E P A E P R P L		25
<hr/>			
rGALR2	. G W Q P E A V L V P L F F A L I F L V G T V G N	I	43
hGALR2	. G W H P E A V I V P L L F F A L I F L V G T V G N		43
rGALR1	F G I G V E N F I T L V F F G L I F F A M G V L G N		50
<hr/>			
rGALR2	A L V L A V L L R G . . G Q A V S T T N L F I L N		66
hGALR2	T L V L A V L L R G . . G Q A V S T T N L F I L N		66
rGALR1	S L V I T V L A R S K P G K P R S T T N L F I L N		75
<hr/>			
II			
rGALR2	L G V A D L C F I L C C V P F Q A T I Y T L D D W		91
hGALR2	L G V A D L C F I L C C V P F Q A T I Y T L D D W		91
rGALR1	L S I A D L A Y L L F C I P F Q A T V Y A L P T W		100



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Figure 15B

		III																								
rGALR2	V	F	G	S	L	L	C	K	A	V	H	F	L	I	F	L	T	M	H	A	S	S	F	T	L	116
hGALR2	V	F	G	S	L	L	C	K	A	V	H	F	L	I	F	L	T	M	H	A	S	S	F	T	L	116
rGALR1	V	L	G	A	F	I	C	K	F	I	H	Y	F	F	T	V	S	M	L	V	S	I	F	T	L	125
rGALR2	A	A	V	S	L	D	R	Y	L	A	I	R	Y	P	L	H	S	R	E	L	R	T	P	R	N	141
hGALR2	A	A	V	S	L	D	R	Y	L	A	I	R	Y	P	L	H	S	R	E	L	R	T	P	R	N	141
rGALR1	A	A	M	S	V	D	R	Y	V	A	I	V	H	S	R	S	S	L	R	V	S	R	N		150	
												IV														
rGALR2	A	L	A	A	I	G	L	I	W	G	L	A	L	L	F	S	G	P	Y	L	S	Y	Y	R	Q	166
hGALR2	A	L	A	A	I	G	L	I	W	G	L	S	L	L	F	S	G	P	Y	L	S	Y	Y	R	Q	166
rGALR1	A	L	L	G	V	G	F	I	W	A	L	S	I	A	M	A	S	P	V	A	Y	Y	Q	R	L	175
rGALR2	S	Q	L	.	A	N	L	T	V	C	H	P	A	W	S	A	P	.	R	R	R	A	M	D	L	189
hGALR2	S	Q	L	.	A	N	L	T	V	C	H	P	A	W	S	A	P	.	R	R	R	A	M	D	I	189
rGALR1	F	H	R	D	S	N	Q	T	F	C	W	E	H	W	P	N	Q	L	H	K	K	A	Y	V	200	



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Figure 15C

	V																										
rGALR2	C	T	F	V	F	S	Y	L	L	P	V	L	V	L	S	L	T	Y	A	R	T	L	R	Y	L	214	
hGALR2	C	T	F	V	F	S	Y	L	L	P	V	L	V	L	G	L	T	Y	A	R	T	L	R	Y	L	214	
rGALR1	C	T	F	V	F	G	Y	L	L	P	L	L	L	L	I	C	F	C	Y	A	K	V	L	N	H	L	225
rGALR2	W	R	T	V	D	P	V	T	A	G	S	G	S	Q	R	A	K	R	K	V	T	R	M	I	I	239	
hGALR2	W	R	A	V	D	P	V	A	A	G	S	G	A	R	R	A	K	R	K	V	T	R	M	I	L	239	
rGALR1	H	K	K	L	K	N	M	S	K	K	S	E	A	.	.	S	K	K	K	T	A	Q	T	V	L	248	
	VI																										
rGALR2	I	V	A	V	L	F	C	L	C	W	M	P	H	H	A	L	I	L	C	V	W	F	G	R	F	264	
hGALR2	I	V	A	A	L	F	C	L	C	W	M	P	H	H	A	L	I	L	C	V	W	F	G	Q	F	264	
rGALR1	V	V	V	V	V	F	G	I	S	W	L	P	H	H	V	I	H	L	W	A	E	F	G	A	F	273	
	VII																										
rGALR2	P	L	T	R	A	T	Y	A	L	R	I	L	S	H	L	V	S	Y	A	N	S	C	V	N	P	289	
hGALR2	P	L	T	R	A	T	Y	A	L	R	I	L	S	H	L	V	S	Y	A	N	S	C	V	N	P	289	
rGALR1	P	L	T	P	A	S	F	F	F	R	I	T	A	H	C	L	A	Y	S	N	S	S	V	N	P	298	



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Figure 15D

rGALR2	I	V	Y	A	L	V	S	K	H	F	F	R	K	G	F	R	K	I	C	A	G	L	L	R	P	A	314
hGALR2	I	V	Y	A	L	V	S	K	H	F	F	R	K	G	F	R	T	I	C	A	G	L	L	G	R	A	314
rGALR1	I	I	Y	A	F	L	S	E	N	F	F	R	K	A	Y	K	Q	V	F	K	C	R	V	C	N	E	223
rGALR2	P	R	R	A	S	G	R	V	S	I	L	A	P	G	N	H	S	G	S	M	L	E	Q	E	S	339	
hGALR2	P	G	R	A	S	G	R	V	C	A	A	R	G	T	H	S	G	S	V	L	E	R	E	S	339		
rGALR1	S	P	H	G	D	A	K	.	.	.	.	.	.	.	.	E	K	N	R	I	D	T	P	P	S	340	
rGALR2	T	D	L	T	Q	V	S	E	A	A	G	P	L	V	P	P	P	A	.	.	.	.	.	.	L	358	
hGALR2	S	D	L	L	H	M	S	E	A	A	G	A	L	R	P	C	P	G	A	S	Q	P	C	I	L	364	
rGALR1	T	N	C	T	H	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	346		
rGALR2	P	N	C	T	A	S	S	R	.	.	.	.	.	.	.	.	.	.	.	T	L	D	P	A	C	372	
hGALR2	E	P	C	P	G	P	S	W	Q	G	P	K	A	G	D	S	I	L	T	V	D	V	A	.	387		

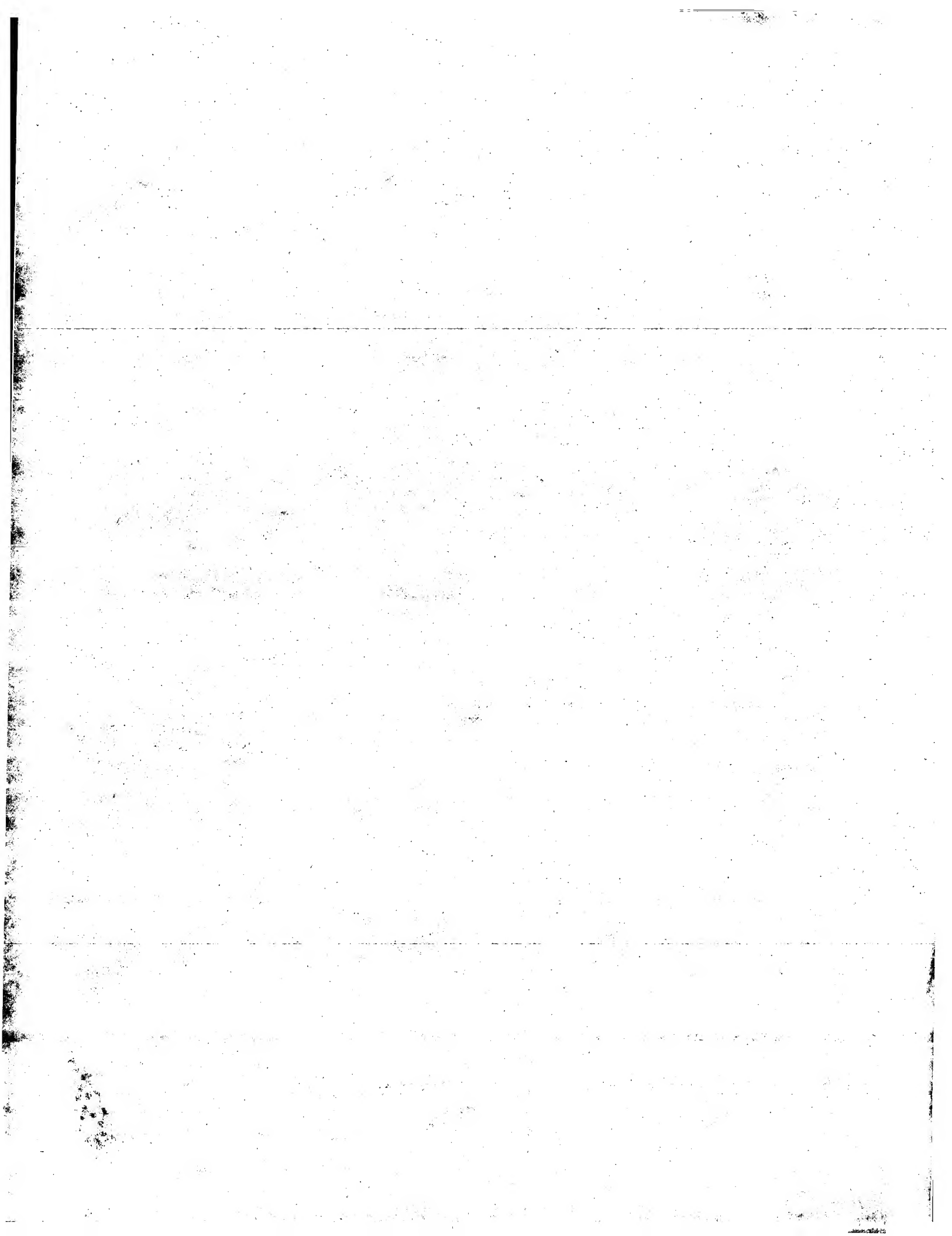






## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

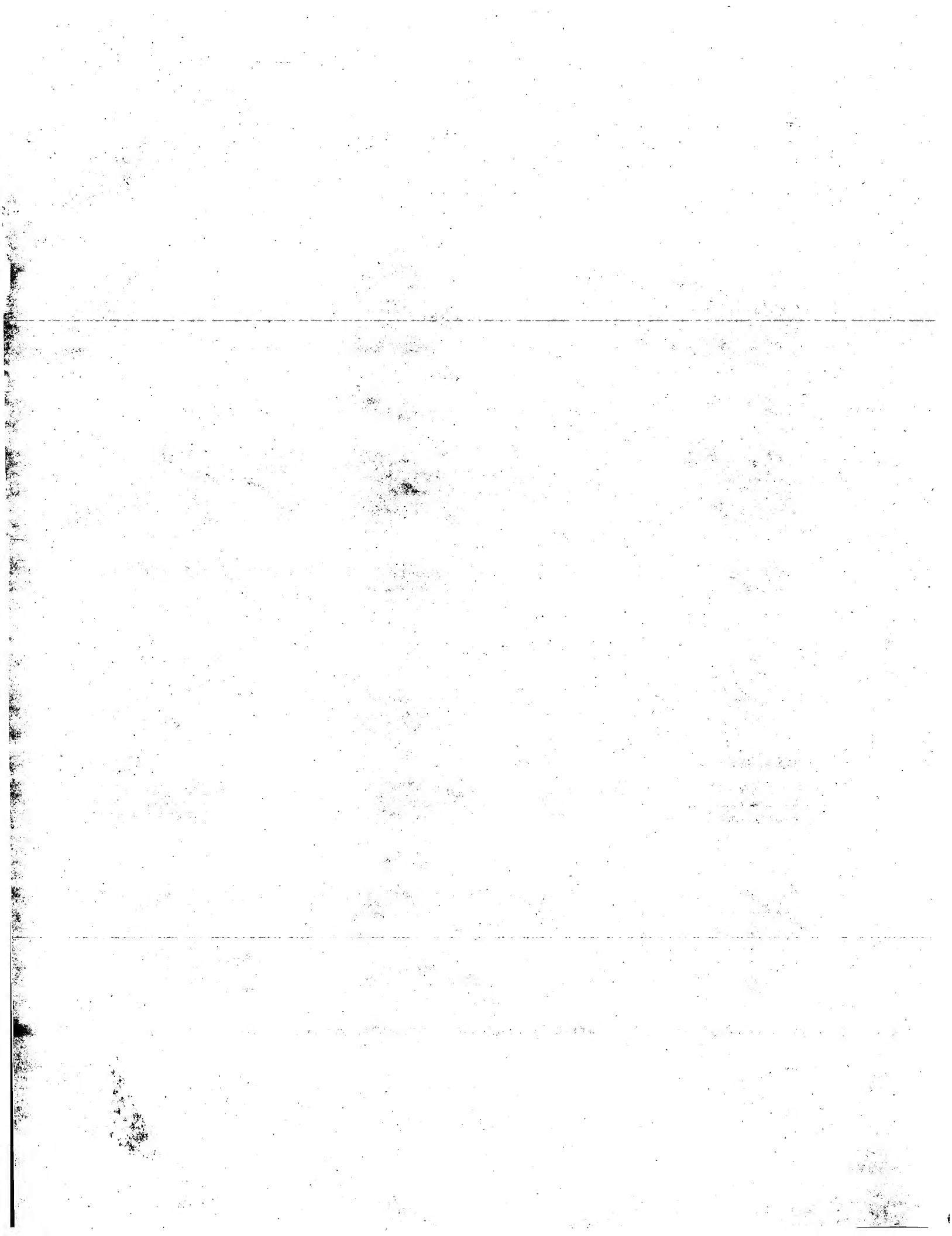
(51) International Patent Classification <sup>6</sup> : C12N 5/10, 15/11, 15/63, 15/70, 15/79, 15/81, 15/85; C07K 14/00; G01N 33/53; C12P 21/00		A3	(11) International Publication Number: <b>WO 97/26853</b>
(21) International Application Number: PCT/US97/01301		(43) International Publication Date: 31 July 1997 (31.07.97)	
(22) International Filing Date: 24 January 1997 (24.01.97)		(72) Inventors; and (75) Inventors/Applicants (for US only): SMITH, Kelli, E. [US/US]; 401 Riverside Drive, Wayne, NJ 07470 (US). GERALD, Christophe, P., G. [FR/US]; 204B Union Street, Ridgewood, NJ 07450 (US). WEINSHANK, Richard, L. [US/US]; 268 Vandelinda Avenue, Teaneck, NJ 07666 (US). LINEMEYER, David [US/US]; 105 Melba Street, Milford, CT 06460 (US). BRANCHEK, Theresa [US/US]; 518 Standish Road, Teaneck, NJ 07666 (US). FORRAY, Carlos [CL/US]; 304 Spring Valley Road, Paramus, NJ 07652 (US).	
(30) Priority Data: 08/590,494 24 January 1996 (24.01.96) US 08/626,046 1 April 1996 (01.04.96) US 08/626,685 1 April 1996 (01.04.96) US 08/721,837 27 September 1996 (27.09.96) US		(74) Agent: WHITE, John, P.; Cooper & Dunham, L.L.P., 1185 Avenue of the Americas, New York, NY 10036 (US).	
(60) Parent Applications or Grants (63) Related by Continuation US 08/721,837 (CIP) Filed on 27 September 1996 (27.09.96) US 08/626,685 (CIP) Filed on 1 April 1996 (01.04.96) US 08/626,046 (CIP) Filed on 1 April 1996 (01.04.96) US 08/590,494 (CIP) Filed on 24 January 1996 (24.01.96)		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): SYNAPTIC PHARMACEUTICAL CORPORATION [US/US]; 215 College Road, Paramus, NJ 07652 (US).		Published With international search report.	
		(86) Date of publication of the international search report: 23 October 1997 (23.10.97)	
(54) Title: DNA ENCODING GALANIN GALR2 RECEPTORS AND USES THEREOF			
(57) Abstract  This invention provides isolated nucleic acids encoding mammalian galanin receptors, isolated galanin receptor proteins, vectors comprising isolated nucleic acid encoding a mammalian galanin receptor, cells comprising such vectors, antibodies directed to a mammalian galanin receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian galanin receptor, antisense oligonucleotides complementary to unique sequences of nucleic acid encoding a mammalian galanin receptor, nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian galanin receptor, as well as methods of determining binding of compounds to mammalian galanin receptors.			



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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/01301

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.2, 69.1, 320.1, 325, 348, 356, 357, 361, 365, 369; 530/350; 536/23.5, 24.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, BIOSCIENCE, BIOSIS, CAPLUS, MEDLINE, EMBASE, SCISEARCH

search terms: galanin receptor, GAL7 receptor

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	PARKER et al. Cloning and characterization of the rat GALR1 galanin receptor from Rin14B insulinoma cells. Molecular Brain Research. 28 December 1995, Vol. 34, No. 2, pages 179-189, especially pages 181-184.	1-3, 5-8, 11, 13, 15, 17, 19, 21, 23, 24, 27, 28-29, 33, 34, 39-40, 42-43, 50, 51-57, 59-60, 81-87, 90-103, 117-122, 147-149, 158-159, 184-185 ----- 31-32, 47-50, 123-128, 150-157

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 21 APRIL 1997	Date of mailing of the international search report 09 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL D. PAK <i>Michael D. PAK</i> Telephone No. (703) 308-0196



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/01301

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 5,436,155 A (BELL et al.) 25 July 1995 (25/07/95), see especially columns 6-7, 14-15, 17-19, 24, and 29-43, and figures 1, and 4-5.	1-9, 11, 13, 15, 17, 19, 21, 26, 27-34, 39, 40, 42-43, 50-60, 81-87, 117-118, 147-149, 158-160, 184-185 ----- 47-50
A	WO 95/22608 A1 (RHONE-POULENC RORER S.A.) 24 August 1995 (24/08/95), see entire document.	1-60, 81-87, 90-103, 117-128, 147-160, 184-220
A	KASK, K. et al. Delineation of the peptide binding site of the human galanin receptor. EMBO Journal. 15 January 1996, Vol. 15, No. 2, pages 236-244, see entire document.	1-60, 81-87, 117-118, 147-149, 158-160, 184-185, 202-205
Y	BOUVIER, M. et al. Dynamic palmitoylation of G-protein-coupled receptors in eukaryotic cells. Methods in Enzymology. 1995, Vol. 250, pages 300-314, especially pages 304-308.	31-32, 47-50
Y, P	US 5,576,296 A (BARTFAI et al.) 19 November 1996 (19/11/96), see especially columns 1, 3-5, and 9-12.	186-201, 206-220
X	US 5,290,808 A (SOFIA) 01 March 1994 (01/03/94), see entire document, especially columns 1-2.	186-201, 206-220
Y, P	US 5,567,714 A (BRUNS, JR. et al.) 22 October 1996 (22/10/96), see especially columns 1-4 and 7-8.	206-217
Y	US 5,436,128 A (HARPOLD et al.) 25 July 1995 (25/07/95), see especially columns 4-5, and 8-12.	123-128, 150-157





# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/01301

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 88-89, 104-112, 129-146, 164-170, 174-181  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  
1-60, 81-87, 90-103, 117-128, 147-160, 184-220
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest



The additional search fees were accompanied by the applicant's protest.



No protest accompanied the payment of additional search fees.



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/01301

## A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):

C12N 5/10, 15/11, 15/63, 15/70, 15/79, 15/81, 15/85; C07K 14/00; G01N 33/53; C12P 21/00

## A. CLASSIFICATION OF SUBJECT MATTER: US CL :

435/7.2, 69.1, 320.1, 325, 348, 356, 357, 361, 365, 369; 530/350; 536/23.5, 24.3

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING (Continued):

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-60, 81-87, 117-118, 147-149, 158-160, 184-185, and 202-205, drawn to a nucleic acid, a GALR2 receptor, a vector, a cell, a membrane preparation, a nucleic acid probe encoding the GALR2 receptor, a process for identifying a chemical compound which binds GALR2, a method of preparing the GALR2 receptor, and a process of determining whether a compound binds GALR2 and another galanin receptor.

Group II, claim(s) 61-63 and 69-73, drawn to an antisense oligonucleotide and a pharmaceutical composition comprising antisense oligonucleotide.

Group III, claim(s) 64-68, and 74, drawn to an antibody and a pharmaceutical composition comprising the antibody.

Group IV, claim(s) 75-80, drawn to a transgenic nonhuman mammal.

Group V, claim(s) 90-103, 119-128, and 150-160, drawn to a process of determining whether a chemical compound is a GALR2 receptor agonist comprising activation of the GALR2 receptor, and a process of determining whether a chemical compound is a GALR2 receptor antagonist.

Group VI, claim(s) 113-114, and 161, drawn to a first compound, an agonist, and a pharmaceutical composition.

Group VII, claim(s) 115-116, 142, and 162, drawn to a third compound, an antagonist, and a pharmaceutical composition.

Group VIII, claim(s) 163, drawn to a method of detecting expression of a GALR2 receptor by detecting the presence of mRNA.

Group IX, claim(s) 186-201, and 206-220, drawn to a method of treating an abnormality in a subject comprising administering an antagonist, a method of decreasing feeding behavior by administering GALR2 antagonist and Y5 receptor antagonist.

Group X, claim(s) 171, drawn to a method of detecting GALR2 with the antibody.

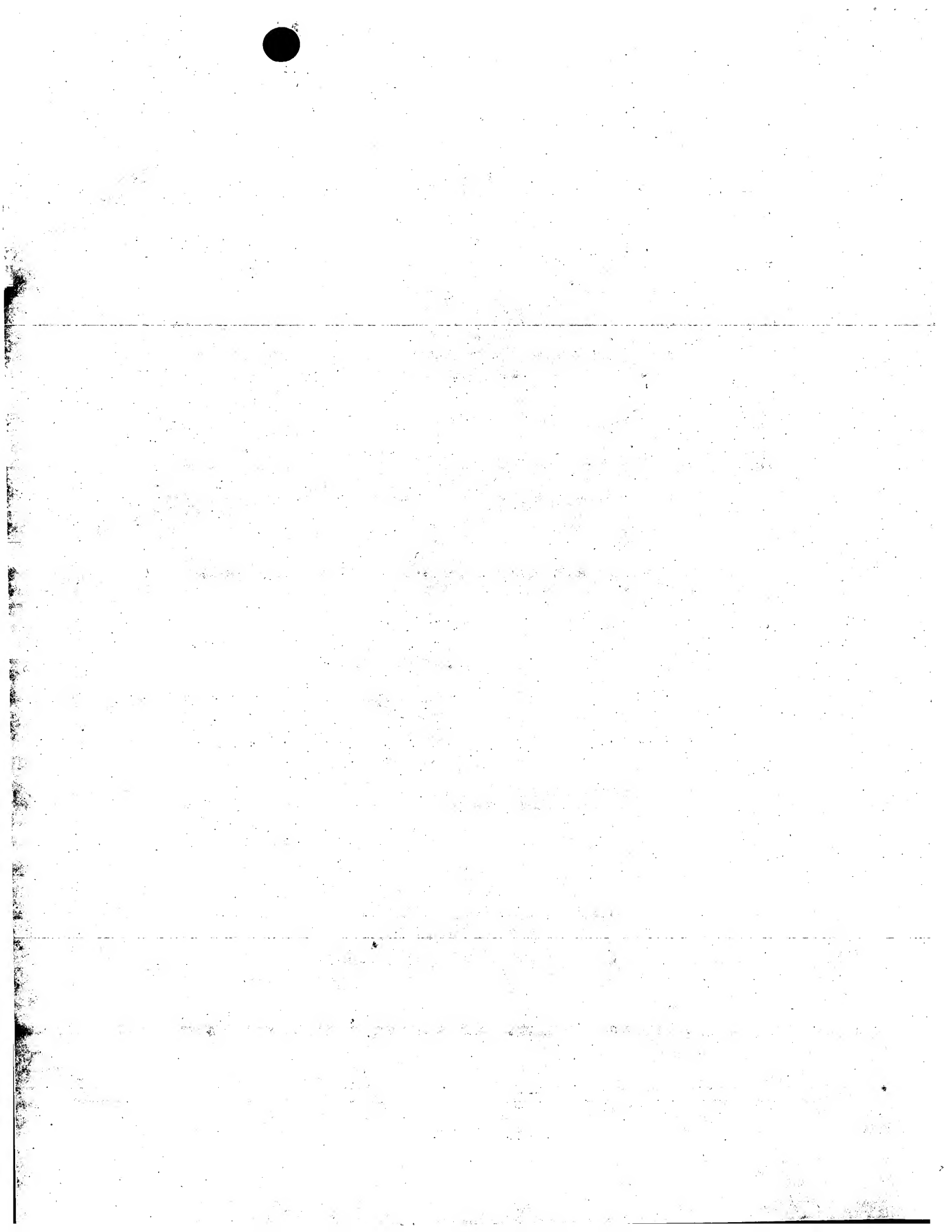
Group XI, claim(s) 172-173, drawn to a method of determining the physiological effects of GALR2 receptor in a transgenic non-human mammal.

Group XII, claim(s) 182-183, drawn to a method for diagnosing a predisposition to a disorder.

and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The inventions listed as Groups I-XII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group I is a nucleic acid, a GALR2 receptor, a vector, a cell, a membrane preparation, a nucleic acid probe encoding the GALR2 receptor, a process for identifying a chemical compound which binds GALR2, and a method of preparing the GALR2 receptor. Pursuant to 37 CFR 1.475(d), these claims are considered by the ISA/US to constitute the main invention, and none of the related groups II-XII correspond to the main invention.



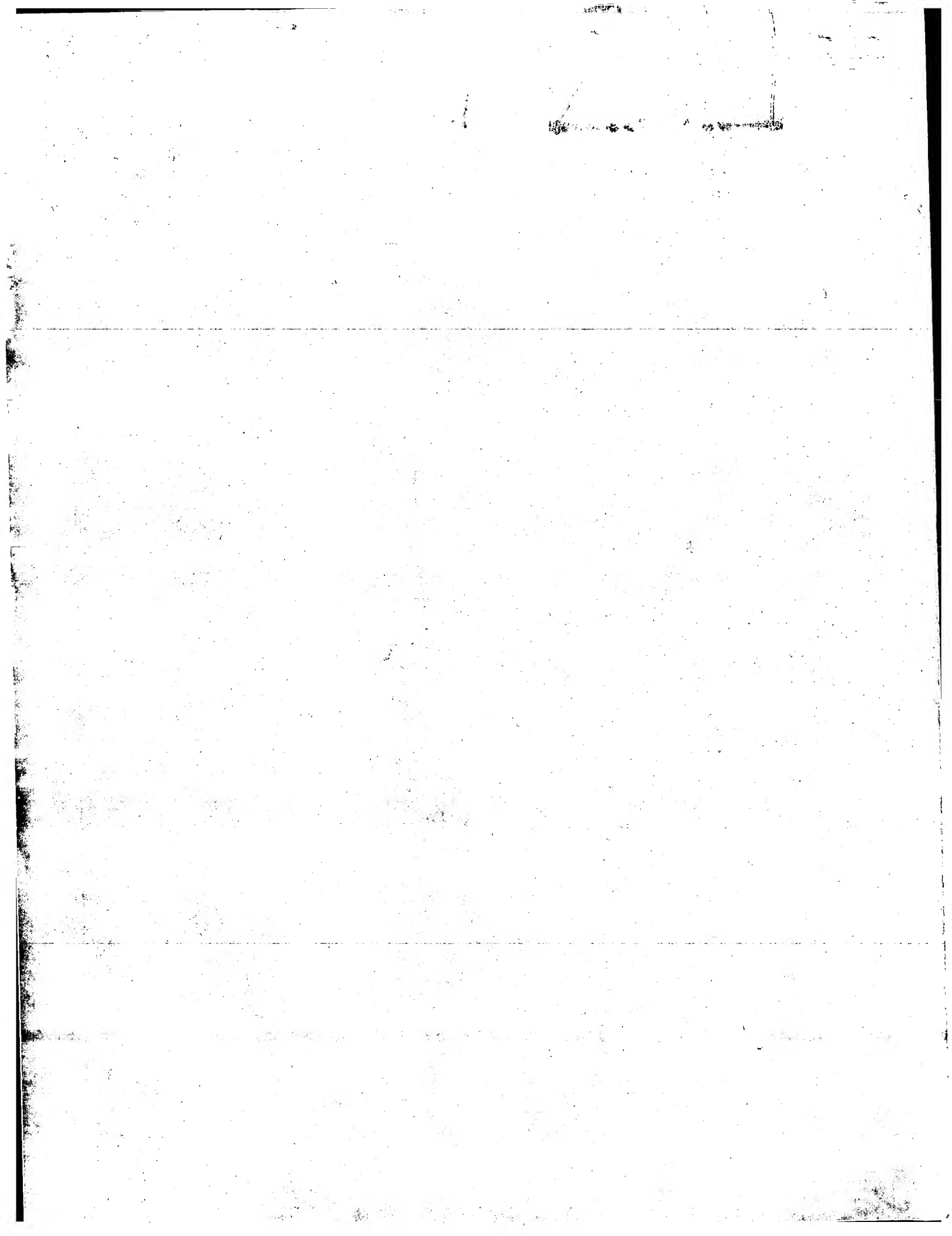
# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/01301

The products of Group II-IV and VI-VII does not share the same or corresponding special technical feature with Group I, because they are drawn to products having materially different structures and functions, and each defines a separate invention over the art.

The methods of Groups V, and VIII-XII, do not share the same or corresponding special technical feature with Group I, because the methods have materially different process steps and are practiced for materially different purposes, and each defines a separate invention over the art.

Since Groups I-XII do not share a special technical feature, unity of invention is lacking.



## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

P,X	FEBS Lett., vol 283, N 2, June 1991, pp 189-194. M. Bersani et al "Human Galanin: primary structure and identification of 2 molecular forms"	1-3, 5-8
X	FEBS Lett., vol 234, No 2, July 1988, pp 400-406. A. Rokaeus and M. Carlquist "Nucleotide sequence analysis of cDNAs encoding a bovine galanin precursor protein in the adrenal medulla and chemical isolation of bovine gut galanin".	1-8
X	Proc. Natl. Acad. Sci. (USA), vol 85, February 1988, pp 1065-9. L.M. Kaplan et al "Tissue-specific expression of the rat galanin gene".	1-8
X	Proc. Natl. Acad. Sci. (USA), vol 83, September 1986, pp 6287-6291. A. Rokaeus and M.J. Brownstein "Construction of a porcine adrenal medullary cDNA library and nucleotide sequence analysis of two clones encoding a galanin precursor".	1-8

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- ☐ Claim numbers, because they relate to subject matter not required to be searched by this Authority, namely:
- ☐ Claim numbers, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
- ☐ Claim numbers, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

- ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
- ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
- ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent classification (IPC) or to both National Classification and IPC Int. Cl. <sup>8</sup> C12N 15/12, C07K 7/10, A61K 37/02, A61K 37/43		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC	Derwent Data base, WPAT: Keywords: human, galanin	
CAS	Keywords: as above;	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>8</sup>		
AU: IPC subclass C12 N 15/12; C07 K 7/10 Biotechnology Abstracts: keywords as above. STN data base: sequence search.		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>9</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No <sup>13</sup>
P,X	Endocrinology, vol. 129, No 3, 1991, pp 1682-1684. H.F. Evans and J. Shine "Human Galanin: Molecular cloning reveals a Unique Structure"	1-9
P,X	Neuroscience Letters, vol 136, 1992, pp 105-108. L.G. Ulman et al "Effects of human, rat and porcine galanins on cardiac vagal action and blood pressure in the anaesthetised cat"	1-3, 5-9
P,X	Proc. Natl. Acad. Sci. (USA), vol 88, Dec. 1991, pp 11435-11439. W.E. Schmidt et al "Isolation and primary structure of pituitary human galanin, a 30-residue nonamidated neuropeptide".	1-3, 5-8
<p>* Special categories of cited documents :<sup>10</sup></p> <p>"A" Document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search 25 June 1992 (25.06.92)	Date of Mailing of this International Search Report 30 June 1992 (30.06.92)	
International Searching Authority  <b>AUSTRALIAN PATENT OFFICE</b>	Signature of Authorized Officer  K. AYERS <i>K. Ayers</i>	